Evolution of Antibiotic Resistance

This unit is actually more of a mini-unit, embedded within a larger unit on evolution, or more specifically, microevolution. The students are challenged with a very topical and relevant problem: the ***growing health crisis of antibiotic resistance***. They first learn about the problem, then investigate the tools they will need to solve the problem (i.e. knowledge of key evolutionary concepts). Using this new knowledge, they come back to the problem, and investigate the causes underlying this problem in depth. Finally, they apply all that they have learned and try to come up with possible solutions to this problem. Approaching the learning of evolutionary concepts in this way will: ***show students the importance of understanding these concepts for real-life problems***, allow students to apply their knowledge to a new situation, and aid in the transfer of student learning of evolutionary concepts to real-life situations.

The unit is organized as follows. First, there is a suggested lesson plan with day-to-day activities and the "big picture." Next, there is background information on each of the specific topics referred to within the activities. Finally, the descriptions for each activity are listed, along with additional instructions for teachers, and worksheets for students.

Suggested uses: This is not intended as a substitute for a broad introduction to evolutionary concepts, but as a supplement to a broad approach. Ideally, this unit should be *embedded within a larger unit on evolution*, in which students learn basic evolutionary concepts (such as variation and natural selection). However, feel free to use individual lessons where they are appropriate within your class. If you have a *microbiology or bacterial unit*, especially if it is after the evolution unit, many of these lessons may be incorporated directly into it, as an applied, real-life example of the concepts they learned about in the evolution unit. **If you use any of this unit in your classroom, please email me (brightk@mso.umt.edu) and let me know how it goes. Any and all feedback is welcome!**

Suggested Lesson Plan for Unit

Part I. Define the Problem to be Investigated: Bacterial Resistance to Antibiotics

Activity A) Investigate Resistance to Antibiotics

Day 1: Have students set up experiment on resistance.

Day 2: Have students examine their experiment and make observations; small group/full class discussion of the significance of their findings.

Activity B) Research a Disease-Causing Bacterium

Day 3: Assign research project, and help students begin researching their bacteria.

Part II. Lessons on Evolutionary Concepts

Introduce students to broad evolutionary concepts (variation, mutation, natural selection, adaptation, speciation, macroevolution, etc.).

Part III. Investigate Reason for Problem 1: Bacteria Evolve!

Activity C) What Is Resistance To Antibiotics?

Day 4: Have students get together in small groups and discuss questions from worksheet; end with a full class wrap-up on what exactly is resistance to antibiotics (an evolutionary response by bacteria).

Part IV. Investigate Reason for Problem 2: Antibiotics Select for Resistance, and Human Behavior Provides Opportunities for Bacteria to Evolve

Activity D) Interpreting Correlational Data

Day 5: Have students break into small groups and work through worksheet; group discussion/lecture on how using antibiotics exerts natural selection for resistance.

Part V. Solutions to the Antibiotics Problem

Activity E) What is the Future of Antibiotics?

Day 5: At the end of class, assign articles to read for the next day.

Day 6: Have students break into small groups to discuss their articles, using the questions in the worksheet as a guide; full-class discussion of possible solutions.

Background Information on Antibiotic Resistance for Teachers

What are Antibiotics?

In the late 1920's, the Scottish microbiologist Alexander Fleming returned from a trip to find that one of his petri dishes containing the bacterium, *Staphylococcus aureus*, was contaminated with the mold, *Penicillium notatum*. Like a good scientist, he did not just throw it out and start over; rather, he made an observation: *there were no bacterial staph colonies growing directly around the mold*. There was a zone that was free of bacterial growth directly surrounding the mold. Upon closer inspection, he noticed that the mold was secreting a liquid (now called penicillin) that he later learned was the cause of death to the bacteria growing in close proximity to the mold.

What Fleming had discovered (actually, re-discovered) was an ***antibiotic***: *a chemical that inhibits the growth of or kills microorganisms (e.g. bacteria).* ***Antibiotics have evolved in fungi and bacteria as defenses against other microbes***. Microorganisms often compete with each other for the same resources. In response to competition, many fungal and bacterial species have evolved chemical weapons to inhibit other species. ***Antibiotics are the chemical weapons of fungi and bacteria***.

What do antibiotics do? How do they kill bacteria? They work through several different means. Some inhibit the synthesis (or production) of bacterial cell walls; some inhibit the synthesis of proteins; still others inhibit the replication of bacterial DNA. All of these are, of course, detrimental to the bacteria that encounter the antibiotic. So detrimental, in fact, it often kills them.

We humans know a good thing when we see it. A chemical substance that kills bacteria: we could use this to our advantage! And did we ever. Within a few decades, both naturally occurring and synthetic antibiotics were produced in mass quantities and given to people who were sick with infectious diseases. And they worked. ***Antibiotics were the miracle cure to all kinds of infectious diseases that had been plaguing humans for hundreds of years***. Antibiotics worked so well, in fact, that in 1969, the U.S. Surgeon General declared: "it is time to close the book on infectious disease." The war against bacteria was over, and we had won!

Or had we?

Antibiotic[1] Resistance

In 1941, all strains of staphylococcal bacteria (common causes of wound and postoperative infection) were ***susceptible*** *to (killed by)* penicillin. Three years later, one

[1] A more general term is antimicrobials; this includes antibiotics as well as drugs that are used to treat diseases caused by microbes other than bacteria (e.g. malaria, HIV/AIDS). Although antibiotics are exclusively discussed here, the evolutionary principles described are similar for all pathogenic microbes, not just bacteria.

strain of staph was no longer susceptible to penicillin; it was ***resistant*** *to (not killed by)* penicillin. Today, especially in hospitals, there are strains of staph bacteria that are resistant to, not just one, but nearly all known antibiotics. Although most of the multiple-drug resistant staph strains are only found in hospitals, recently, four children in North Dakota and Minnesota were killed by staph infections that they had acquired outside of a hospital.

Staphylococcus is not the only problem bacterium. More than two-dozen types of bacteria are now resistant to one or more types of antibiotics that had previously been effective against them. Certain strains of three bacterial species (*Enterococcus*, *Pseudomonas*, and *Mycobacterium tuberculosis)* are resistant to every antibiotic, including vancomycin, the antibiotic previously known as "the drug of last resort." Multi-drug resistant tuberculosis (TB, caused by *Mycobacterium tuberculosis*) is nearly epidemic in some areas of the world (e.g. Russia)[2].

Antibiotics are not as effective at killing bacteria as when they were first introduced. People are dying from infections that were easily treated just a few years ago. It has been estimated that infections caused by resistant bacteria kill as many as 77,000 people every year in the United States alone. Resistance to antibiotics costs dollars as well as lives: it costs the nation up to $30 billion every year.

What happened? Why can we no longer cure infections that were very easily treated just a few years ago?

What is Resistance to Antibiotics?

There are several ways to address this question. First of all, there is a prevalent misconception that antibiotics no longer work because the people who take the drugs have developed a tolerance for the drug. This is not the case. ***Humans do <u>not</u> develop a tolerance for antibiotics***. Antibiotics work by inhibiting or killing the bacteria living inside of us. The reason they no longer work (i.e. we do not get better after taking the antibiotic) is that the bacteria are no longer inhibited/killed by the drug—they are resistant to the effects of the antibiotic.

So, back to our question, what is resistance to antibiotics? Let us first address this question *physiologically*. There are several ways that bacteria resist the effects of antibiotics. Some resistant bacteria inactivate the antibiotic by destroying or modifying the drug itself so that it is no longer toxic. Some resistant bacteria pump the drug out of the bacterial cell so that the concentration of the drug is too low to be effective. Still, other resistant species have an altered form of the target site of the drug (the place on the cell where the drug binds), so the antibiotic cannot "find" its target. These are examples of the types of *resistance characters* that bacteria use to fight antibiotics.

Now, let us address this question on a different level: *evolutionarily*. What has happened to make these bacteria resistant to antibiotics? Have individual bacteria developed

[2] For other examples of the growing problem of antbiotic resistance, see NIH fact sheet (www.niaid.nih.gov/factsheets/antimicro.htm)

a tolerance to the drug? Have they physiologically acclimated to the presence of the antibiotic so that it no longer affects them? No. What has happened is bacterial evolution. **M*utations that allow the bacteria to resist the effects of the antibiotic occur and have a selective advantage***. These mutations have the type of effects that were described in the previous paragraph (for example, there is a mutation that results in an altered form of the target site). These resistance characters are often simple mutations (i.e. changes in a single gene). The result is that ***resistant bacteria differ genetically from their susceptible ancestors***.

So what happens if a bacterial cell has a mutation that allows it to resist the effect of an antibiotic? If that bacterium is in the presence of the antibiotic, then it will have an advantage: the drug will not kill it! It will be able to reproduce, while the susceptible bacteria (which are inhibited or killed by the antibiotic) will not**. *In the presence of the antibiotic, the resistant mutant has a selective (reproductive) advantage over normal cells***[3]. Originally, most or all bacteria in the population were susceptible to the antibiotic[4]. Over many generations, the resistant type will make up a greater and greater percentage of the population. Eventually, most or all of the individuals in the bacterial population will be resistant to the antibiotic. The population has evolved resistance due to natural selection by antibiotics: the genetic structure of the population has changed, from susceptible to the antibiotic to resistant to the antibiotic.

Why Does Resistance Evolve so Quickly?

Bacterial populations can evolve resistance very quickly. For example, in one hospital, initially 5% of the strains of staphylococcal bacteria were resistant to the antibiotic ciprofloxacin. Within one year, 80% of the bacterial strains were resistant. From 5% to 80% in one year! Why do bacterial populations evolve resistance so quickly? There are two basic reasons:

1) **in general, bacteria have the capacity to evolve quickly**
2) **humans are helping them to evolve even faster**

Bacteria Biology

There are several aspects of bacteria biology that contribute to their capacity for rapid evolution. Bacteria, relative to humans, have very **short generation times**. A generation

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[3] Note: when these mutations occur in the absence of the antibiotic, the resistant form does not have a selective advantage, and evolution of resistance does not occur. Typically, these resistance characters are evolutionarily costly to the bacteria that have them (e.g. they must divert energy from reproduction to maintaining the resistance character), so when these mutations occur in the absence of the antibiotic, they are selected against.

[4] There may also be a very small percentage of the original population that is naturally resistant to the antibiotic. Naturally resistant bacteria are partially resistant—they are not be killed by a low dosage of the antibiotic, but will be killed by a higher dosage. This is analogous to humans and the flu—not everyone gets the flu, even if they are exposed to it (some people are naturally resistant). Naturally resistant bacteria were present long before the first antibiotics were used in humans (a legacy of the age-old arms race between competing microorganisms). Since the heavy use of antibiotics in humans, they have become much more common, and mutations that strengthen this partial resistance have been selected for.

time is the time it takes to go from one generation to the next. For example, in humans, it takes on average about 20 years to go from the birth of a child to the birth of that child's child. Therefore, the generation time for humans is approximately 20 years. Contrast this with the average bacterial generation time of hours or even minutes! Under favorable conditions, a single bacterial cell will very quickly reproduce into a colony containing many generations of its offspring and their offspring. These colonies can have so many individual cells that, within hours or days, it will be large enough to see with the naked eye. ***Organisms with fast generation times, like bacteria, have the capacity for very rapid adaptation to a changing environment***. Since evolutionary change occurs across generations, organisms with fast generation times (like bacteria) can evolve much faster than organisms with slow generation times (like humans). Some bacteria species can go through thousands of generations in a single year.

Bacterial populations are also very high in numbers and are quite genetically variable. Mutations are the primary source of genetic variation. Mutations (accidents in DNA replication) are rare events. In bacteria, a mutation at a particular gene occurs on average once in about every 10,000,000 cell divisions. Since bacteria are so numerous and divide so often, even these rare events actually occur quite often. As an example, *E. coli* cells in a human colon divide 2×10^{10} times every day. That means that every day in an *E. coli* population, approximately 2000 cells will have a mutation at a particular gene[5]. So, even though mutations are rare events, they occur often enough in bacterial populations to create a lot of genetic variation within populations.

Mutation is not the only way that a bacterium can acquire a resistance gene. Bacteria have three other **methods of acquiring genes** that sexual organisms (like us) do not have. Bacteria can pick up pieces of DNA (containing genes) from their environment (*transformation*), they can obtain a gene from another bacterium (*conjugation*), and genes can also be transferred to a bacterium by a virus (*transduction*). So, even if a resistance gene does not occur through mutation, it can be acquired through one of these methods.

To summarize, ***bacterial populations evolve resistance to antibiotics so quickly because of their fast generation times, large population sizes, and unique methods of gene acquisition***. These are some of the reasons that bacteria have been so evolutionarily successful.

Human Behavior

The second reason that bacterial populations evolve resistance to antibiotics so quickly is that ***several aspects of human behavior actually contribute to their capacity to evolve rapidly***. Understandably, when antibiotics first became available, people started to use them. A lot. Today, **antibiotics are overused**, and unfortunately **antibiotics are often misused**.

[5] If the probability of a mutation at a particular gene is 1×10^{-7}, and there are 2×10^{10} cell divisions, then the average number of cell divisions that will result in a mutation at this gene is: $(1 \times 10^{-7}) * (2 \times 10^{10}) = 2000$.

Overuse:

- ? It has been estimated that nearly half of all medical prescriptions for antibiotics in the U.S. are unnecessary. Many doctors prescribe antibiotics under pressure from their patients, even if the antibiotic is not warranted (e.g. for a viral infection). Direct-to-consumer marketing by pharmaceutical companies can also lead to inappropriate demand for antibiotics by patients.

- ? Almost half of all antibiotics produced in North America and Europe are given to livestock; most are given not to fight infection, but prophylactically to promote growth in healthy animals. There is growing evidence that this use of antibiotics in livestock leads to resistance in human bacteria.

- ? It is currently trendy to include antibacterial agents in common household cleaning products (even hand lotion!). It is becoming more and more difficult to find cleaners without antibacterial agents.

Misuse:

- ? Medical doctors, including veterinarians and dentists, often incorrectly prescribe antibiotics: they prescribe the wrong antibiotics or the incorrect dosage of an antibiotic for a particular infection; they prescribe antibiotics for non-bacterial infections (e.g. colds, coughs, or influenza); they prescribe antibiotics prophylactically (in a low dosage for months at a time to prevent future infections; for example, for young children with a history of multiple ear infections).

- ? Many doctors also prescribe broad-spectrum antibiotics, which kill many different types of bacteria, rather than run a diagnostic lab test so they can prescribe a narrow-spectrum antibiotic that would specifically target the bacteria causing the infection.

- ? In many other countries, antibiotics are freely available over the counter, without a doctor's prescription, leading to widespread misuse.

- ? Patients themselves also contribute to the problem when they feel better after a few days, and then stop taking the antibiotics, instead of continuing with the full cycle prescribed to them. In a 1995 Gallup poll, it was estimated that more than half of American adults taking antibiotics failed to complete their prescribed dosage.

Compounding all of these problems, the pharmaceutical industry (until very recently) had all but stopped research and development of new antibiotics.

Bacteria Biology + Current Human Behavior = Fast Evolution

How have these two factors helped speed up the evolution of resistance? In essence, ***we are exerting extremely strong selection pressures on these bacteria by our heavy use of antibiotics***. Bacteria are continuously exposed to antibiotics, and this has created very strong selection on these populations to evolve resistance. The more exposure to antibiotics that bacterial populations have, the greater the selection pressure on these populations to evolve

resistance. The rate that evolutionary change occurs depends directly upon the strength of natural selection imposed. Strong selection leads to rapid evolution.

Antibiotics do not just kill the bacteria species that we want them to act on (i.e. the bacteria causing the infection we are trying to get rid of). ***Antibiotics also affect a lot of bacteria that are beneficial to us, or that are commensal with us (neither harmful nor beneficial).*** This decreases the population sizes of these other bacteria, which reduces the competition for the harmful bacteria that survive. This lack of competition for resources allows the surviving resistant bacteria to do very well.

In addition, ***by using antibiotics incorrectly, we are giving the bacterial populations the opportunity to adapt quickly***. For example, if you take an antibiotic correctly—in an adequately high dosage and for the entire cycle—most of the bacteria in your system will be killed. By greatly reducing the population size of the bacteria, you greatly decrease the chance that any one bacterium will mutate to a resistant form. However, if you incorrectly take the antibiotic—if you stop taking it after a few days or if the dosage is not high enough—more of the bacteria will survive[6]. Higher numbers of bacteria means a greater chance that a resistance mutation will occur in any one of the bacterial cells. When these mutations do occur, they rapidly increase in the population, due to the very strong selection pressure exerted by the presence of the antibiotic.

In conclusion, the combination of several aspects of bacterial biology (fast generation time, high population sizes) and human behavior (heavy use of antibiotics, misuse of antibiotics) has led to an ever-increasing problem of bacteria resistant to our only means of controlling them.

What is the Future of Antibiotics?

Can we stop the evolution of resistance? Because of their quick generation times and high numbers, bacteria have a very high capacity to quickly adapt to changing environments. We cannot change the biology of the bacteria. ***As long as we expose bacteria to antibiotics, they will evolve resistance to them. However, we can slow down the evolution of resistance by modifying human behavior.***

What can be done to slow down the evolution of resistance?

First, decrease the selection pressure on bacterial populations by decreasing the overall use of antibiotics. Researchers are recommending prudent use of antibiotics (see website of the Alliance for the Prudent Use of Antibiotics). Doctors and patients need to be educated about when and how to use antibiotics appropriately. Antibiotics should not be prescribed for viral infections, such as the common cold. For minor bacterial infections, a period of "watchful waiting" for a day or two to see if the infection will clear on its own has also been recommended.

[6] Also, any naturally resistant bacteria will survive if the antibiotic is not taken correctly (see footnote 4).

Scientists are also recommending that the agricultural industry discontinue the use of antibiotics in livestock and on crops, especially those antibiotics that are used to treat disease in humans (see website of the Union of Concerned Scientists).

Second, stop giving bacteria extra opportunities for mutations (i.e. use antibiotics appropriately). When an antibiotic is necessary, the most appropriate antibiotic should be prescribed based on the results of laboratory tests to confirm the precise bacterium causing the infection. Often, a doctor will prescribe an antibiotic without conducting a laboratory test to determine the bacterial species causing the infection. If the antibiotic is not appropriate, and the patient does not get better, he/she then comes back for a different prescription. In this case, all of the bacteria in the patient were unnecessarily exposed to an inappropriate antibiotic. When possible, narrow-spectrum antibiotics should be used, rather than a broad-spectrum antibiotic, which affects many different types of bacteria.

Antibiotics need to be taken in strong enough dosages to kill all the bacteria causing the infection, and they need to be taken responsibly: each dose should be taken on time, and all doses (i.e. the full cycle) should be taken. Doctors and pharmacists should be educated about responsible usage, and they should actively encourage their patients to take antibiotics responsibly—exactly as prescribed, and for the entire course. Patients should not demand antibiotics from their doctors.

Third, reduce the spread of resistant bacteria from one person to another. This can be done with the same techniques used for controlling the spread of diseases themselves—better hygiene, clean water, vigorous hand washing, etc. The agricultural industry can also help to stop the spread of resistant bacteria by not using the same antibiotics in animals that are also used in humans (to avoid, for example, transferring resistant bacteria to humans in the food that we eat).

Finally, more research is needed. Research on the optimal use of antibiotics will be necessary. It is still unclear exactly how to decrease the selection pressure on bacterial populations. The above suggestions can only help, but more research about how bacterial populations respond to antibiotics is still needed. Also needed is basic research on microbial biology: physiology, genetics, ecology, and evolution. Understanding basic biological processes in these organisms will help to develop new drugs and treatment protocols.

Most "new" antibiotics these days are modified from older ones. Because these drugs are so similar to older varieties, resistance evolves very quickly. Research and development of completely new antibiotics will also become increasingly important. Pharmaceutical companies have started to respond to this need, but since it can take up to ten years for a new drug to be approved for use in the United States, there will be a lag time before new drugs will become available.

Summary and Conclusions

To summarize, antibiotics have been miracle drugs, curing all sorts of formerly incurable infectious diseases. Unfortunately, humans have used these drugs unwisely,

allowing the bacteria to quickly evolve ways to get around them. If we had been aware of the principles of evolutionary biology, if we had understood that the heavy use of antibiotics applies enormous selection pressure on bacterial populations to evolve resistance, and that incorrect usage of antibiotics gives the bacterial populations the opportunity to quickly evolve resistance, then we may not have found ourselves in the situation that we are today. Granted, even if antibiotics had been used wisely from the very beginning, bacterial populations would still have evolved resistance eventually; however, it would have taken much longer, we would have known it would happen, and would have been better prepared to manage the evolution of resistance. ***Unfortunately, because we ignored evolution for so long, we are in a crisis of antibiotic resistance***.

References Used:

Campbell, N.A., J.B. Reece, & L.G. Mitchell. 1999. Biology (5th ed.). Benjamin/Cummings, California.

Daniel, H.J. III 1999. Otitis media: an evolutionary perspective, in W.R. Trevathan, E.O. Smith, & J.J. Mckenna (eds.) Evolutionary Medicine. Oxford University Press, New York.

Ewald, P.W. 1994. Evolution of Infectious Disease. Oxford University Press, Oxford.

Falkow, S. & D. Kennedy. 2001. Antibiotics, Animals, and People—Again! Science 291: 397.

Levin, B.R. & R.M. Anderson. 1999. The population biology of anti-infective chemotherapy and the evolution of drug resistance: more questions than answers, in S.C. Stearns (ed.) Evolution in Health & Disease. Oxford University Press, Oxford.

Nesse, R.M. & G.C. Williams. 1994. Why We Get Sick: The New Science of Darwinian Medicine. Vintage Books, New York.

Neu, H.C. 1992. The crisis in antibiotic resistance. Science 257: 1064-1073.

Nissinen, A., P. Grönroos, P. Huovinen, E. Herva, M. Katila, T. Klaukka, S. Kontiainen, O. Liimatainen, S. Oinonen, & P.H. Mäkelä. 1995. Development of ?-lactamase-mediated resistance to penicillin in middle-ear isolates of Moraxella catarrhalis in Finnish children, 1978-1993. Clincal Infectious Diseases 21: 1193-6.

Palumbi, S.R. 2001. The Evolution Explosion: How Humans Cause Rapid Evolutionary Change. W.W. Norton & Company, New York.

Radetsky, P. 1998. Last days of the wonder drugs. Discover, November, 1998.

Rose, M.R. 1998. Darwin's Spectre: Evolutionary Biology in the Modern World. Princeton University Press, New Jersey.

Seppälä, H., T. Klaukka, J. Vuopio-Varkila, A. Muotiala, H. Helenius, K. Lager, P. Huovinen, and the Finnish Study Group for Antimicrobial Resistance. 1997. The effect of changes in the consumption of macrolide antibiotics on erythomycin resistance in group A streptococci in Finland. New England Journal of Medicine 337: 441 – 446.

Williams, R.J. & D.L. Heymann. 1998. Containment of antibiotic resistance. Science 279: 1153-115.

Web resources on antibiotic resistance:

Alliance for the Prudent Use of Antibiotics: www.healthsci.tufts.edu/apua/index.html

Centers for Disease Control and Prevention: www.cdc.gov/drugresistance/index.htm *and*

www.cdc.gov/ncidod/emergplan/antiresist/page_3.htm

National Institutes of Health: www.niaid.nih.gov/factsheets/antimicro.htm

Union of Concerned Scientists: www.ucsusa.org/index.html

U.S. Food and Drug Administration: www.fda.gov/oc/opacom/hottopics/anti_resist.html

World Health Organization: www.who.int/emc/amr.html

Part I: Define the Problem

Activity A) Investigate Resistance to Antibiotics—Teacher's Guide

Overview: Begin the evolution unit with a real problem: bacterial resistance to antibiotics.
Learning Objectives: Students will understand what antibiotics are and what they do (i.e. they are chemicals that kill bacteria, which humans have co-opted and use to fight bacterial infections); students will understand that antibiotics do not kill all of the bacteria they are used against.
Class Time: 2 – 3 days
Day 1: Have students set up experiment on resistance.
Day 2: Have students examine their experiment and make observations; small group/full class discussion of the significance of their findings.
Background Information: "What are Antibiotics?" and "Antibiotic Resistance"
Materials: petri dishes filled with nutrient agar (one or two for each student)
sterile cotton swabs
distilled water (or tap water, if distilled water is unavailable)
antibiotic disks (several kinds, one disk of each per student)
control disks (one per student)
markers
scotch tape (to seal the lid of the petri dish to the bottom, for transport)
copies of worksheet (one for each student)

In this exercise, students will be introduced to simple methods used in microbiology. Students collect bacteria from the classroom or themselves, and test these bacteria against several antibiotics. They will see "first-hand" the effects that antibiotics have on bacteria, as well as the ability of some bacteria to resist antibiotics. This will lead into a lecture/discussion on antibiotics, resistance to antibiotics, and the increasingly serious public health crisis.

Materials can be obtained from a biological supply catalog. Blank control disks and antibiotic disks are typically sold in packs of 50. I would suggest you *use several different types of antibiotics* that have been very commonly used; including both antibiotics that work on gram positive bacteria and antibiotics that work on gram negative bacteria; for example, *erythromycin, penicillin, streptomycin, and tetracycline*. Tetracycline has been used a lot for skin infections (e.g. acne), so this would be a particularly good one to use if you have students swab the oily parts of their faces. If you do not have microbiology materials in-house, you can also obtain petri dishes prepared with nutrient agar from these sources.

Encourage students to *collect bacteria from a variety of sources* (each student choosing one source only). Sink drains, or other moist places work well, as do places with a lot of dust. They could even swab themselves (e.g. the oily skin around their nose or forehead).

How long it takes for visible growth of the colonies will depend on the environmental conditions where they are stored. Ideally, place the petri dishes in an incubator set at 37^{o} C,

but if that is not available, set them on a warm shelf, *away from direct sunlight*. If you do not have an incubator and your room is not particularly warm, you might do the first part on a Friday, and the second part the following Monday.

Points for Discussion:

- ? Be sure to discuss why they are using a control (blank) disk.
- ? Ask students why they think some bacteria can grow closer to the antibiotic disks than other bacteria can.
- ? Review the importance of mutation as a source of variation.
- ? Review ecological concepts (population growth, etc.) so that students understand what they are seeing.

Investigate Resistance to Antibiotics—Student Worksheet

In this laboratory investigation, you will investigate the effect of antibiotics on bacteria.

Part I: Collect and culture bacteria.

Materials:
petri dish with nutrient agar
sterile cotton swab
distilled water
one control disk
antibiotic disks (one of each type)
marker
scotch tape

Instructions:

1) **Collect bacteria:**
   - ? choose a location to collect bacteria
   - ? wet a sterile cotton swab with distilled water (or tap water, if distilled water is not available)
   - ? thoroughly rub the swab around your chosen place

   Record the location you collected your sample: _____

2) **Spread collected bacteria over the medium:**
   - ? rub the cotton swab back and forth across the agar (covering the entire plate)
   - ? rotate the plate 90^o(1/4 turn), and rub the swab back and forth across the entire plate
   - ? rotate the plate another 90^o (in the same direction), and repeat.

3) **Place the disks on medium**
   - ? *carefully place a control disk (a "blank" disk with no antibiotic) onto the medium and press it gently into the medium (*Why do you place the control disks first, before the treated disks?*)
   - ? carefully place each of your antibiotic disks onto the medium, gently pressing them into the medium
     *note: be sure to evenly space the disks across the medium, none of them too close to the edge (see figure)
     *note: as you place each disk, be sure to label the **bottom** of the petri dish (i.e. the side with the nutrient agar) with the name of the antibiotic at that spot

4) **Incubate the dish**
   - ? close the lid on the petri dish
   - ? use scotch tape to seal the lid of the petri dish to the bottom
   - ? store the dish agar side (bottom) up in an incubator

Part II: Make observations.

Check on your culture daily, and make observations, using the following questions to guide you. You may use a dissecting microscope, if available. Describe what you see in words and make detailed drawings. You do not have to be an "artist" to make scientific drawings; these are descriptive drawings, and are used everyday by scientists to make detailed observations.

1) Describe and draw any bacterial or fungal colonies you see. How many different types are there? What color are they? What kind of texture do they have (smooth, shiny, dull, wet, dry, waxy, etc.)? How big are they? What shape are they? Are they raised or flat against the surface of the agar?

2) Draw and describe any "zones of inhibition" surrounding the disks (an area without bacterial growth). Which disks have zones around them? How big is each zone? Are there different zones for different types of bacteria? Or for different types of antibiotics? Are there any bacterial colonies growing within a zone?

Activity B) Research a Disease-Causing Bacterium—Teacher's Guide

Overview: Continue the investigation of the problem: bacterial resistance to antibiotics.
Learning Objectives: Students will understand that resistance to antibiotics is a growing, very serious, public health problem.
Class Time: 1 day
Day 3: Assign research project, and help students begin researching their species.
Background Information: "What are Antibiotics?" and "Antibiotic Resistance"

In this assignment, students will investigate the problem of antibiotic resistance on their own. They are assigned one species of bacteria to research: what disease(s) it causes, what antibiotic(s) have been used against it, and whether or not those antibiotic(s) are still effective. This is a very flexible assignment. It could be done as a group project or an individual assignment. It could be a written assignment and/or a short oral presentation (depending upon how much class time you have, and how it fits in your class). The worksheet "Research a Disease—Student Instructions" is just one suggestion for how to assign this project.

Be sure to help students with how to conduct research. For example, give them reputable websites (see list below) and aid them in computer searches for articles. Tell them the difference between peer-reviewed, primary literature (journal articles), secondary literature (books and reviews of the primary literature), and websites (for the most part, non-peer reviewed, and should be taken with a grain of salt, unless it is maintained by a reputable organization). Also instruct students on how to cite references, both in-text, and at the end of the paper in a "literature cited" section (see "**A Brief Guide to the Literature**").

Suggested bacterial species:
Mycobacterium tuberculosis
Staphylococcus aureus
Enterococcus faecalis
Pseudomonas aeruginosa
Streptococcus pneumoniae
Escherichia coli
Streptococcus faecalis

Web resources on antibiotic resistance:
Alliance for the Prudent Use of Antibiotics: www.healthsci.tufts.edu/apua/index.html

Centers for Disease Control and Prevention: www.cdc.gov/drugresistance/index.htm *and* www.cdc.gov/ncidod/emergplan/antiresist/page_3.htm

National Institutes of Health: www.niaid.nih.gov/factsheets/antimicro.htm

Union of Concerned Scientists: www.ucsusa.org/index.html

U.S. Food and Drug Administration: www.fda.gov/oc/opacom/hottopics/anti_resist.html

World Health Organization: www.who.int/emc/amr.html

A Brief Guide to the Literature

Primary literature includes peer-reviewed articles in scholarly journals, such as Nature, Bioscience, Science, etc. ***Secondary sources*** are books or edited volumes that use the primary literature (e.g. textbooks).

When you use an idea that you obtained from one of your sources, cite this source in the text, *using the last name of the author(s) and the publication year in parentheses*. For example:

… (Chen 1994)…

If you use the author(s) name(s) as part of your sentence, then just include the publication year in parentheses:

Rudolph and Stewart (1998) …

For citations with more than two authors, just list the first author's name and et al.

…(Morris et al. 1997)…

At the end of the paper, include a **Literature Cited** section, with the full reference of each of the sources you cite within your text (in alphabetical order by author). The following are examples of full citations from a *book, and a journal article*:

Nesse, R.M. & G.C. Williams. 1994. Why We Get Sick: The New Science of Darwinian Medicine. Vintage Books, New York.

Neu, H.C. 1992. The crisis in antibiotic resistance. Science 257: 1064-1073.

Websites are generally not peer-reviewed and thus are not primary or even secondary literature. When citing websites, just give the address of the site within the text (in parantheses). If you need to include websites in the literature cited (in most cases, you will not need to), give a very brief description of the site, as well as the address. For example:

Centers for Disease Control and Prevention
http://www.cdc.gov

Research a Disease—Student Instructions

For this assignment, you will conduct library research on a specific bacterium that causes a disease in humans, and prepare a two-paged, typed report. Specifically, you will investigate the history of the disease caused by your bacterium, and speculate on its future.

Use the following questions to guide you (but do not just list the answers in your report!): What kind of disease does this bacterial species cause? What would your chance of survival have been if you caught this disease 100 years ago? 30 years ago? Today? 10 years from now? Be sure to explain why you think this. What antibiotics have been used on this disease? Are they all still effective? Will they be effective 10 years from now? Explain why you think this.

Be sure to cite all references you used.

Part II. Evolutionary Concepts

Overview: Introduce students to broad evolutionary concepts (variation, mutation, natural selection, adaptation, speciation, macroevolution, etc.).

Learning Objectives: Students will understand basic evolutionary concepts; students will obtain the tools needed to fully understand the antibiotic problem.

Class Time: 1 – 2 weeks

Part III. Investigate Reason for Problem 1

Activity C) What is Resistance to Antibiotics? — Teacher's Guide

Overview: After students are familiar with key evolutionary concepts, make the connection between these concepts and resistance to antibiotics.

Learning Objectives: 1) Students will understand that bacterial populations evolve resistance to antibiotics. 2) Students will understand that mutations for resistance that occur in the presence of an antibiotic will have a selective advantage, leave more offspring, and thus increase in number in the population. 3) Students will understand that scientific claims are based on carefully collected evidence.

Class Time: 1 day

Day 4: Have students get together in small groups and discuss questions from worksheet; end with a full class wrap-up on what exactly is resistance to antibiotics (an evolutionary response by bacteria).

Background Information: " What is Resistance to Antibiotics?"

In this exercise, students are challenged to apply the evolutionary concepts they have learned to the practical problem they are faced with: antibiotic resistance. The objective is to understand what bacterial resistance is (evolution of bacterial populations) and what it is not (the development of tolerance by individual bacteria or people).

Have students work in pairs or small groups (of 3 – 4). Armed with their research on antibiotics and disease (Part I) and their new knowledge about evolutionary concepts (Part II), have them work through the worksheet together. Use the points for discussion with individual groups, or as a full-class wrap-up to assess student understanding of the key concepts.

Answers to student questions:

Question 2: These data rule out hypothesis A, since they provide evidence that the bacteria have changed (the person has not changed).

Question 3: These data support hypothesis C, but do not rule out hypothesis B, since more and more of the population could be developing a tolerance for the drug.

Question 4: These data support hypothesis C, and rule out hypothesis B, since there is evidence of a genetic difference between the susceptible and resistant bacteria. The population has clearly evolved: most likely, a mutation occurred in a gene affecting the target site of the antibiotic, and since the population was in the presence of the antibiotic, this mutant was selected for and increased in the population.

Points for discussion:

? make sure students understand that a **mutation** occurred, and since it was advantageous in the presence of the antibiotic (it was not killed by the antibiotic), it had a reproductive advantage, and thus increased in the population.

? be sure that students are aware of the difference between **develop** (a non-genetic change that occurs within an individual) and **evolve** (a genetic change that occurs within a population of individuals across generations). These two terms are very different biologically, but are often used interchangeably, especially in the popular press (e.g. "bacteria develop resistance").

What is Resistance to Antibiotics?—Student Worksheet

In this task, you are challenged to understand exactly what is going on in a bacterial population that is now resistant to an antibiotic to which it had previously been susceptible. Work through the following questions.

1) You are infected with a bacterial disease. Your sister had this same illness last week, and took a full cycle of antibiotics. She quickly became better. You started taking the same antibiotic, but they had no effect. In fact, you had to return to the doctor after a week, because you did not feel better. What has happened? Why did you remain sick after taking antibiotics, while your sister quickly recovered? There are three possible hypotheses:

   A) you developed a tolerance for the antibiotic (i.e. you experienced a non-genetic change that made you less sensitive to the effects of the antibiotic).
   B) the bacteria infecting you developed a tolerance for the antibiotic (i.e. individual bacteria experienced a non-genetic change that made them less sensitive to the effects of the antibiotic).
   C) the bacteria infecting you evolved to be resistant to the antibiotic (i.e. a genetic mutation for resistance occurred in a bacterial cell, it had a reproductive advantage and increased in the population).

   a) Which hypothesis (A, B, or C) do you think is most likely? _____
   b) Explain why you chose this one.

2) When you first visited your doctor, she told you that she is conducting research on antibiotics, and asked you to be a part of the study. You agree. As part of the study, you go to the doctor every day and let her take a new sample from your infection, which she then conducts tests on. She discovered that on the first day, your bacteria were susceptible to the antibiotic (i.e. the bacteria were killed by the antibiotic). She then prescribed the antibiotic to you, which you immediately began taking. Later in the week, the bacteria from your infection were found to be resistant to the antibiotic (i.e. the bacteria were not killed by the antibiotic).

   a) This result rules out which of the three hypotheses (A, B, or C)? _____
   b) Why does this result rule out this particular hypothesis?

3) Another result from the study is that initially, all the bacteria were susceptible to the antibiotic, but by the third day, some of the bacteria were resistant to the antibiotic. With each passing day, more of the bacteria were resistant, until finally all of the bacteria were resistant.

   a) Does this result support either (or both) of the remaining hypotheses? _____
   b) Does it allow you to rule out either of them? _____
   c) Explain your answers to a) and b).

4) Your doctor performs a DNA analysis of the bacteria causing your infection, and discovers that the resistant bacteria differ from the susceptible bacteria by one gene: the gene that encodes the protein on the bacterial cell that is the "target" of the antibiotic (the target site is the place in the bacterial cell where the antibiotic binds and does its dirty work). The resistant bacteria have an altered form of this target site, with the result that the antibiotic is unable to bind to the target site, and thus is unable kill the bacterium.

   a) How did the resistant bacteria come to be different genetically from their susceptible ancestors?

   b) Which hypothesis does this result support? _____
   c) Why does this result support this particular hypothesis?

5) Given all of the above evidence, which hypothesis (A, B, or C) do you think is most likely correct? Explain.

Part IV. Investigate Reason for Problem 2

Activity D) Interpreting Correlational Data—Teacher's Guide

Overview: Students will continue to investigate the reasons behind the growing crisis in antibiotic resistance, focusing more on how human behavior is aiding the evolution of antibiotic resistance.

Learning Objectives: Students will understand that the use of antibiotics exerts selection pressure on bacterial populations

Class Time: 1 day

Day 5: Have students break into small groups and work through worksheet; group discussion/lecture on how using antibiotics exerts natural selection for resistance.

Background Information: "Why does Resistance Evolve so Quickly?"

The goal of this lesson is to get students to think about the use of antibiotics as the actual cause of the evolution of resistance. ***Use of antibiotics creates selection pressure on the bacterial populations*** exposed to the antibiotics, thus leading to the evolution of resistance. In the exercise, students examine real data on the amount of antibiotics used in one community over time, and the prevalence of resistance to the antibiotic over the same time period. The amount of antibiotics used is positively correlated with the level of resistance over this time period. You can also use these data to show the ***gradual evolution from no resistance to increasingly more and more resistance***.

The data were taken from a community in Finland from 1978 – 1993. The researchers collected data on the annual amount of ? -lactam (penicillin, ampicillin, and related) antibiotics used in this community. They also collected samples of the bacterial species *Moraxella catarrhalisis* from young children with middle ear infections. They then examined the bacterial strains to see if they were susceptible or resistant to these antibiotics. The data given are a standardized measure of the volume of antibiotic usage (calculated as average daily dose), and the percentage of the bacterial strains that were found to be resistant to the antibiotic (between 0 – 100%). Data were taken from Nissenen et al. (1995) and Levin & Anderson (1999). The data from question 4 are also real (Seppälä et al. 1997); use of the antibiotic erythromycin was greatly limited in a Finnish community, with the result that the percentage of group A streptococci resistant to it was cut in half.

Suggested Uses:

Have the students graph the raw data, or give them copies of the following sample graphs. Have them make two graphs (antibiotic usage vs. year and percent resistant strains vs. year) and/or have them plot a correlation: percent resistant strains vs. antibiotic usage (with each point on the graph being the data for both for the same year). This will show them a positive correlation between these two variables.

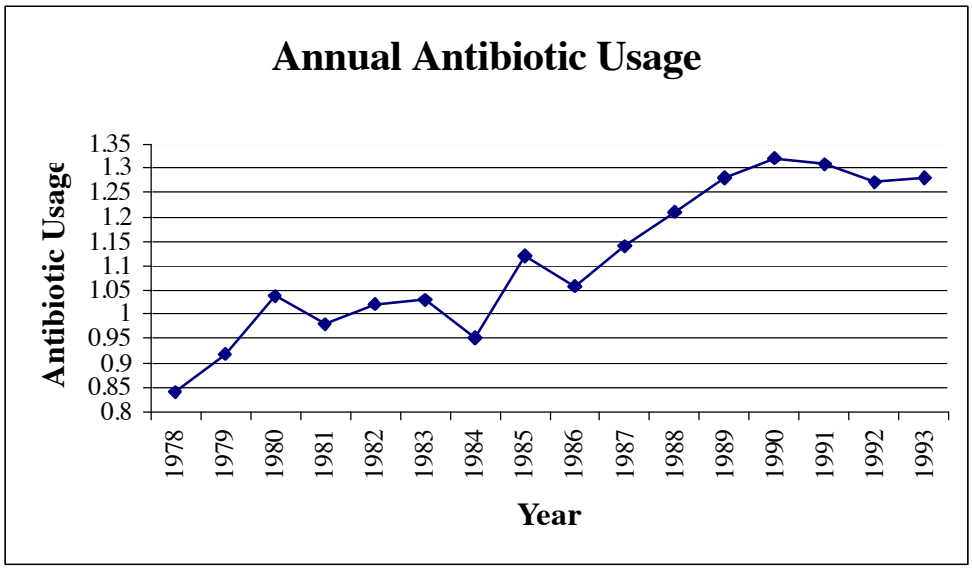
Points for Discussion:

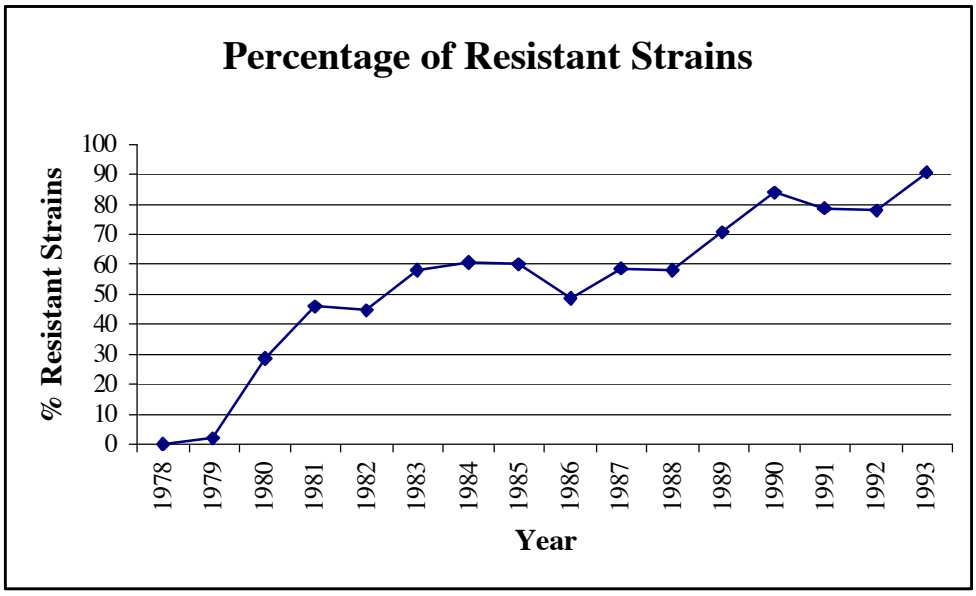
? Of course, *correlation does not equal causation*, and this is important to point out to your students. But, correlative data such as these, along with other evidence (e.g. resistant bacteria are able to survive and thrive in the presence of antibiotics) allows us to conclude that the use of antibiotics are selecting for the evolution of resistance to them. Use question 3 to get this point across. The data in question 4 provides further evidence that antibiotic usage selects for resistance: *when the antibiotic use is limited, selection for resistance decreases, thus leading to the evolution of decreased resistance*.

Literature Cited:

Levin, B.R. & R.M. Anderson. 1999. The population biology of anti-infective chemotherapy and the evolution of drug resistance: more questions than answers, in S.C. Stearns (ed.) Evolution in Health & Disease. Oxford University Press, Oxford.

Nissinen, A., P. Grönroos, P. Huovinen, E. Herva, M. Katila, T. Klaukka, S. Kontiainen, O. Liimatainen, S. Oinonen, & P.H. Mäkelä. 1995. Development of ?-lactamase-mediated resistance to penicillin in middle-ear isolates of Moraxella catarrhalis in Finnish children, 1978-1993. Clincal Infectious Diseases 21: 1193-6.


Annual Antibiotic Usage
Antibiotic Usage
1.35
1.3
1.25
1.2
1.15
1.1
1.05
1
0.95
0.9
0.85
0.8
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
Year


Percentage of Resistant Strains
% Resistant Strains
100
90
80
70
60
50
40
30
20
10
0
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
Year

Interpreting Data—Student Worksheet

In this exercise, you will interpret real data on antibiotic resistance. These data were collected from a community in Finland from 1978 – 1993. The researchers collected data on the annual amount of antibiotics used in this community. They also collected samples of bacteria from young children with middle ear infections. They then examined the bacterial strains to see if they were susceptible to (killed by) or resistant to (not killed by) these antibiotics. The data given below are a measure of the amount of antibiotics used each year, and the percentage of the bacterial strains that were found to be resistant to the antibiotic (between 0 – 100%).

Year	Annual Antibiotic Usage	Percent Resistant Strains
1978	0.84	0
1979	0.92	2
1980	1.04	29
1981	0.98	46
1982	1.02	45
1983	1.03	58
1984	0.95	61
1985	1.12	60
1986	1.06	49
1987	1.14	59
1988	1.21	58
1989	1.28	71
1990	1.32	84
1991	1.31	79
1992	1.27	78
1993	1.28	91

Instructions:
Graph these data in two different graphs: A) **antibiotic usage vs. year** (antibiotic usage on the y-axis, year on the x-axis) and B) **percent resistant strains vs. year** (percent resistant strains on the y-axis, year on the x-axis). Using this information, answer the following questions:

1) Overall, what happened to the annual antibiotic usage in this community over the period of the study (did it increase, decrease, or stay the same)?

_____

2) Overall, did the percentage of bacterial strains resistant to these antibiotics increase, decrease, or stay the same over this same time period?

_____

3) a) From these data, do you think these two factors (antibiotic usage and percentage of strains resistant to the antibiotics) are related? _____

b) Do you think one of these factors was the cause of the other factor? _____

c) If you do, which one? _____

d) How might you be able to tell for sure?

4) In another study, also in Finland, researchers investigated the effect of greatly limiting the use of an antibiotic on a community (kids still got ear infections, but they now used a "wait and see" approach to see if the infection would clear up on its own). After antibiotic usage was greatly decreased, the percentage of bacterial strains that were resistant to this antibiotic was decreased by 50% (cut in half). What does this new information tell you?

Part V. Solutions to the Antibiotic Problem

Activity E) What is the Future of Antibiotics? — Teacher's Guide

Overview: Students will apply their new knowledge of evolutionary concepts, as well as their knowledge of the antibiotics problem and its causes to discuss possible solutions.
Learning Objectives: Students will understand that understanding evolution and evolutionary concepts is critical to identifying possible solutions to this growing health problem.
Class Time: 1 day
(*Day 5:* At the end of class, assign articles and worksheet.)
Day 6: Have students break into small groups to discuss their articles, using the questions in the worksheet as a guide; full-class discussion of possible solutions.
Background Information: "What is the Future of Antibiotics?"

In this exercise, students will discuss potential solutions to the antibiotic problem. They will begin by reading an article (as a homework assignment), and come to class prepared to discuss the article (have the students fill out the worksheet as they are reading the assigned article). The overall learning objective is for students to understand that understanding evolution and evolutionary concepts is critical to identifying possible solutions to this growing health problem. The article is used as a jumping-off point for discussion. The discussion should be student-oriented, with the teacher guiding the discussion towards higher thinking objectives (analysis and synthesis).

There are several ways to approach this. You could have each student read the same article, or you could assign several articles, one to each student, in a jigsaw-type exercise. In this case, within each small group of 3-4 students, each student would be the "expert" on their article, summarizing the article for the other students. This method may be preferable, since no one article lists all possible solutions.

As a spin-off, you might ask students to think about how the recent anthrax scare, and consequent stockpiling of cipro by individuals may affect our ability to respond in the case of an actual bioterrorism event. See the web site of the Alliance for the Prudent Use of Antibiotics (www.healthsci.tufts.edu/apua/index.html) for a discussion of this problem and related links.

Suggestions for Articles:

Braffman-Miller, J. *Beware the rise of antibiotic-resistant microbes*. USA Today Magazine. March 1997.
[This article is a bit long, but has a good overview of the history of antibiotics, the problem of antibiotic resistance (including a "close to home" discussion of a TB outbreak in a California high school), the reasons for the problem (including an explicit discussion of evolution), and potential solutions (vaccines, limiting use in humans, new antibiotics.) This would be a good article if you feel your students need the overview]

Christensen, D. *Keeping bugs from pumping drugs*. Science News. Feb. 12, 2000.
[This article details efflux pump inhibitors, which inhibit bacteria from pumping antibiotics out of their cells—one type of resistant mechanism. This drug may give new life to antibiotics that are now useless because of resistant bacteria. These drugs are also mentioned in Travis' article.]

Levy, S.B. *The challenge of antibiotic resistance*. Scientific American, March 1998.
[This article is long, but written by a leading researcher in the field of the evolution of antibiotic resistance (one of the researchers interviewed in Radetsky's article). It discusses the problem of resistance, as well as a problem that is often overlooked: the affect of antibiotics on the benign bacteria that normally help keep pathogenic bacteria in check. It discusses ways to reverse resistance (i.e. solutions to the problem), and also includes a box on the potential effects of antibacterials in household products.]

Nemecek, S. *Beating bacteria: new ways to fend off antibiotic-resistant pathogens*. Scientific American. Feb.,1997.
[This is a very short article that basically lists several novel methods researchers are using to combat resistant bacteria, including genomics, new natural resources, synthetic chemicals, and bispecific antibodies.]

Radetsky, P. *Last days of the wonder drugs*. Discover. Nov. 1998.
[This is a bit long, but a very good article. It explicitly deals with the problem of antibiotic resistance, and a potential solution based on evolutionary biology (including interviews with two evolutionary biologists working on this problem). The solution discussed is prudent use, with the hope of reverting bacterial populations to susceptibility.]

Travis, J. 1994. *Reviving the antibiotic miracle?* Science 264 (5157): 360.
[Despite being in Science, this is actually a review article that discusses new research into fighting bacteria. It describes several novel approaches, including "disarming" bacteria, rather than killing them, and new drugs, such as chemicals that block the pumps that resistant bacteria use to remove antibiotics from cells.]

Points of Discussion/Questions to Guide Discussion:

? As long as we use antibiotics against bacteria, they will evolve resistance. The question is, how can we manage/slow down this process? Since human behavior (overuse and misuse of antibiotics by industry, doctors, patients) is speeding up the evolution of resistance, how can we try to change human behavior to slow this evolutionary process down?

? Be explicit about how understanding evolution is critical in identifying solutions (although implementing these solutions is a different question—see next point).

? In addition to the medical/scientific aspects of this problem, you may also want to discuss political and economic aspects as well (e.g. what will decreasing the use of antibiotics in agriculture do to productivity? how do we implement programs to effect change in our country, as well as encourage it in other countries?)

? Be sure to cover the following points:

- decrease natural selection on bacterial populations by decreasing the overall amount of antibiotics used (i.e. prudent use)
- stop giving bacterial populations opportunities to evolve: take full cycle of antibiotics, etc.
- reduce the spread of bacteria that are already resistant
- more research: basic research on microbial biology and development of new drugs

What is the Future of Antibiotics?—Student Instructions

Read your assigned article for homework. During the next class, we will be discussing potential solutions to the problem of antibiotic resistance. As you read your article, answer the following questions:

1) While reading the article, identify and define any words that were unfamiliar to you.

2) Summarize the three or four main points the author tries to make in the article.

3) What does the author view as the problem(s) with antibiotics?

4) What does the author suggest as solutions to these problems?

5) Can you think of any other solutions?