Super Bug: Antibiotics and Evolution

by
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Part I – The Rise of the Super Bug

I was sitting in a hospital bay and I wasn't really worried. I get urinary tract infections all the time. It happens when you have Berger's disease, a type of auto-immune thing that affects the kidney, so that you have to get dialysis to do what the kidneys are supposed to do. So getting urinary track infections is no big deal. I had been to the doctor a couple of times already, but the pills he gave me didn't seem to be working. I was already here for my dialysis treatment, so I figured maybe the hospital doctors could give me something more powerful to help clear up the infection faster. I expected to get some IV antibiotics, sit around bored, and then be on my way. I was just visiting my cousins in India. Maybe I should have taken their offer and gotten some powerful antibiotics. It still amazes me that you don't need a prescription to get them over there; it would've made my life easier if they were so easy to get at home.

A nurse comes in briskly. "We're going to move you to your own room, honey."

I should be excited. You know, my own TV without all the noise and craziness just being in a curtained bay. But the nurse looks worried and she is pushing my bed along kind of fast. There is a ton of activity in the hospital bay I left as I see the other patients in the neighboring bays are being moved as well. What weirdness is this about? I'm getting a little worried.

"The doctor will be with you shortly," she says. But I don't believe that. Doctors take forever. That's why I am surprised when he comes along 10 minutes later.

"Ok, ahh… Sam," he says, consulting my chart. "We got your test results back and it looks like your bacterial infection is very serious."

Ok, now I am really worried and maybe even a little bit scared because I just don't understand why this time is different and worse than the others.

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Yeah, so I guess I was the first U.S. patient with the New Delhi Super Bug. Instead of my normal stay in the hospital, I was there for weeks. I barely got to see anyone since I was quarantined. According to the doctors, I probably picked up the bacteria when I was getting dialysis at the hospital on my vacation in India. I really don't understand why my infection was a super bug. What exactly is a "super bug"? What makes infection with a super bug different from my other infections?

While I sat there in the hospital room, I couldn't watch any more daytime television, so I decided to do a couple of internet searches. I mean really; I'm not a pre-med student for nothing. So, Wikipedia first…it seems that a super bug is just a hyped up way of saying that the drugs used to treat the infection don't. These "super bugs" apparently have antibiotic resistance. Here's one of the "visuals" I found:

Exposure to Antibiotics

Increasing Antibiotic Resistance
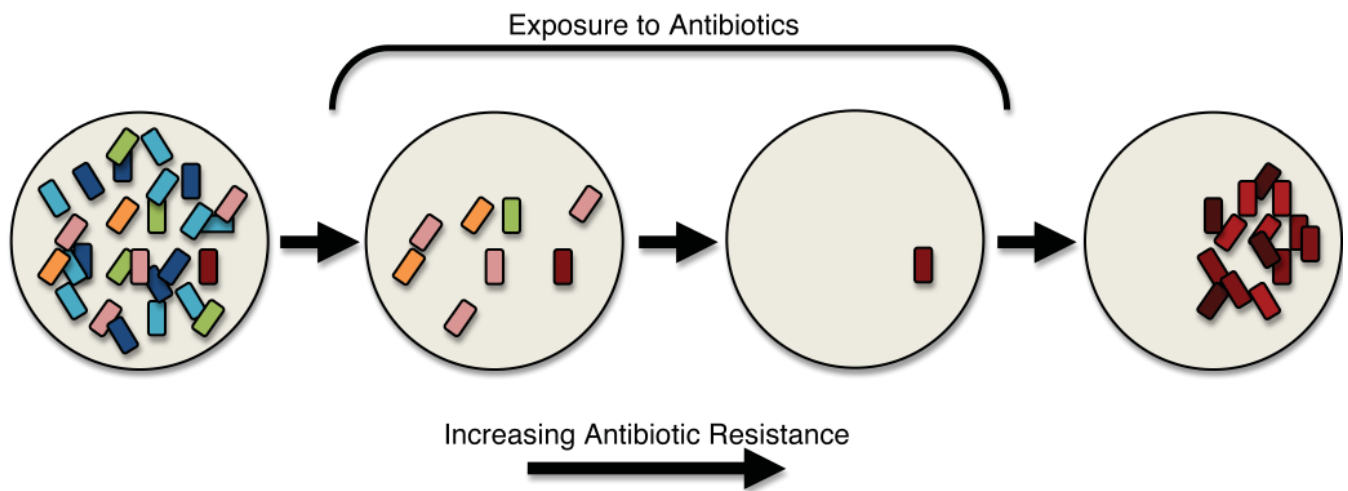
So while I thought this information on antibiotic resistance was helpful, what exactly does this have to do with me sitting here forever? I Googled "Super Bug" and found a variety of news stories. Here's an "extract" from one of them:

> "Doctors in India aren't surprised that the Super Bug probably originated there. Drug control there is poor and common antibiotics have become ineffective in India. Some reasons may be because people can buy powerful antibiotics over the counter, leading to overuse. They also take small doses and discontinue treatment in order to save money. There are no current antibiotics, nor any in development, that can kill New Delhi Super Bug on their own." –Vinny Ciancio (AccessRx)

Questions

1. What do you think could allow some of the bacteria to live even in the presence of antibiotics? (Hint: Are all the bacteria in a population the same? How might they differ?)
2. What are the biological, social, or cultural factors that may have influenced the increased resistance of this strain of bacteria to antibiotics? (i.e., what could have influenced the development of New Delhi Super Bug and influenced its spread?)

Exercise

The acquisition of antibiotic resistance in certain bacterial species in a given location occurs over time. How does antibiotic resistance relate to evolution?

Form a group of 4 students. Define each of the principles of evolution listed below. Then compare them to Sam's story of the antibiotic resistant bacteria. Explain how bacterial antibiotic resistance demonstrates each principle. Designate 1 student in the group to report your findings to the rest of the class.

- Phenotype vs. genotype
- Diversity
- Allele and allele frequency
- Gene pool
- Selection or natural selection
- Population bottleneck
- Heritable traits
- Adaptation
- Population vs. individual
- Mutation
- Lateral gene transfer

Part II – Mechanism of Evolution

"I know what a super bug is," I muttered to myself. "It's simply a bacterium that is resistant to most or all of the drugs that could be used to treat it." The boredom of just sitting here was really getting to me, so I kept reading my Google search results to find out how exactly a bacteria becomes resistant to antibiotics. Here's some of what I found:

> "Experts say that the infection is worth taking seriously, but the public health implications are uncertain. Multi drug-resistant bacteria have been around for decades. In fact, the so called "super bug" is not actually a bug at all. Instead, the drug resistance comes from a gene called NDM-1 that gets passed from one kind of bacteria to another. These genes have sparked even more concerns because they don't lie in the genomes of the bacteria themselves. Instead, they sit on small, circular pieces of DNA called plasmids, which can be passed between bacteria.
>
> What sets NDM-1 apart is that it lies on a plasmid that seems to move especially freely between bacteria, even if they are completely unrelated. That means the gene could move from an *E. coli* bacterium that causes urinary tract infections to other types of bacteria that cause pneumonia, salmonella, or cholera. With some 100 trillion bacteria on and in our bodies at all times, plasmids with the NDM-1 gene have many opportunities to spread. That can happen inside a person's gut, in the soil, or within water that contains fecal contamination." —Emily Sohn *(Discovery News)*

Wow, the whole idea of bacteria passing DNA back and forth for antibiotic resistance is scary. One author I read described the situation perfectly as an "ongoing arms race between the bacteria that cause illnesses and the drugs designed to kill them."

Since I had already done some reading about antibiotic resistance, I decided to write a paper on antibiotic resistance for my genetics class. Below are two pieces of data from primary research articles on NDM-1 or urinary tract infections that I found to use as references.

Your Task

Examine the graphs and figures associated with the two experiments below and then answer the questions that follow.

Experiment 1

Scientists examined how much antibiotic it would take to kill the bacterium *E. coli,* with and without the NDM-1 gene. The NDM-1 gene encodes an enzyme that breaks down antibiotics rendering them inactive. A graph of their results is presented to the right.

The x-axis represents the specific antibiotic tested as well as the class of antibiotic that it belongs to. Each class of antibiotics has similar chemical structure and mechanism of action. For example, penicillin inhibits remodeling of the peptidoglycan cell wall of bacteria, which weakens the cell wall, making them susceptible to be lysed. In contrast, macrolides inhibit bacterial protein synthesis by binding irreversibly to the 50S subunit of the ribosome, inhibiting translocation of peptidyl-tRNA.
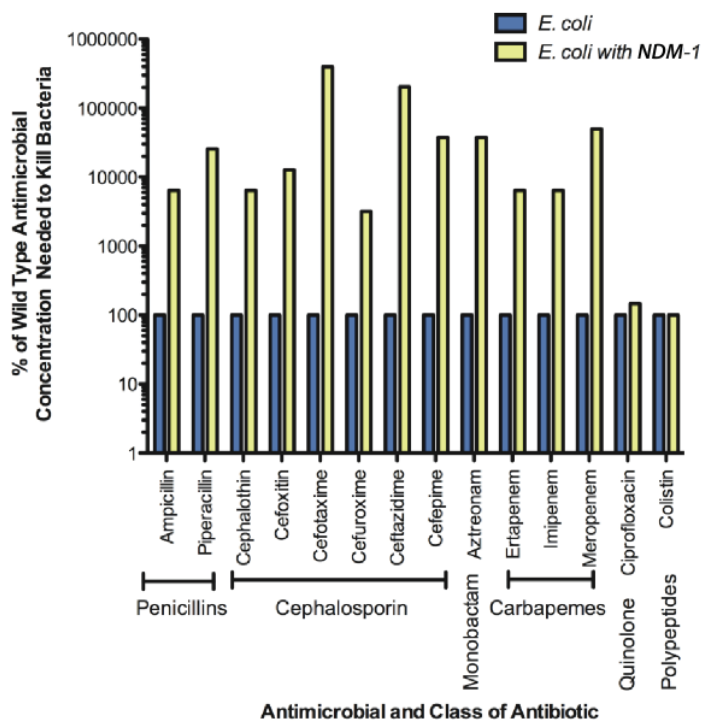
The y-axis represents the amount of antibiotics needed to kill the bacteria. It is represented as a percentage of the amount needed to kill the *E. coli* that does not contain NDM-1 gene (wild type). Thus for all the antibiotics, wild type is 100%. For example to kill the wild type bacteria it takes 1 μg/mL Piperacillin; in contrast, to kill *E. coli* with NDM-1 it takes over 256 μg/mL Piperacillin. Therefore, the percentage of wild type to kill the *E. coli* with NDM-1 is 25,600%. Please note that the y-axis is represented on a log scale.

Experiment 2

The mechanism of action of different antibiotics varies, but the quinolone class of antibiotics acts to prevent DNA replication. For bacteria to replicate their DNA, they need to separate the strands of DNA from each other. Two key enzymes help accomplish this task: DNA gyrase *(gyrA)* and topoisomerase IV *(parC)*.

Over a 14-year period, 828 *E. coli* isolates were collected from patients with urinary tract infections (UTI) similar to Sam's. Then these isolates were examined for quinolone-specific resistance and the presence of mutations in the *gyrA* and *parC* DNA sequences that gave *E. coli* quinolone resistance.

The first table below describes these results from the randomly chosen isolates. The sequences of the *gyrA* and *parC* genes were known, so the sequences of the isolates were compared to the known sequence. The mutations found are described in the chart by the amino acid encoded by unmutated DNA, the position of the amino acid, and the amino acid that the DNA was mutated to. For example, *gyrA* has a Serine at position 83 with a DNA sequence corresponding to a TCA codon. The sequence of an isolate at the same position in the DNA was TTA. This is a codon for Leucine instead of Serine. Then the percentage of quinolone-resistant bacteria that contained this mutation is listed.

gyrA				parC			
Mutation			Resistant Isolates Containing Mutation	Mutation			Resistant Isolates Containing Mutation
Start Amino Acid	Position	Final Amino Acid		Start Amino Acid	Position	Final Amino Acid	
Serine	83	Leucine	100%	Serine	80	Arginine or Isoluecine	93.6%
Aspartic Acid	87	Glycine or Asparagine	98.7%	Glutamic acid	84	Valine or (Glycine, Alanine, Lysine)	56.4%

Notice that the isolated resistant bacteria contain a very high percent of each mutation. Below is a table describing the relationship between the number of mutations in these two genes compared to resistance to quinolones where the percentage is the number of bacteria of each type that have that number of mutations. For example, of the 9 isolates of antibiotic susceptible bacteria, 55.6% or 5 had no mutations.

Mutation Number	Susceptible (9 isolates)	Resistant (78 isolates)
0	55.6%	
1	22.2%	1.3%
2	22.2%	2.6%
3		42.3%
4		53.8%

Questions

1. Examine the graph from experiment 1.
   a. How many times more ampicillin does it take to treat *E. coli* that has NDM-1? (Hint: Convert the % of wild type to how many more times ampicillin is needed.)
   b. Would taking this much more be reasonable? (Consider that a normal dose is 1 pill.) How many pills would you have to take?

2. According to experiment 2, how does the number of mutations affect resistance to quinolones?

3. How do you think the mutations in *gyrA* and *parC* cause resistance to quinolones? (Hint: Look at the amino acid numbers and remember that drugs need to bind the target.)

4. Which of the following would be more likely to increase the resistance of bacteria to more than one class of antibiotics: the mutations in *gyrA* and *parC,* or the expression of NDM-1? Why?

5. Explain the order of events concerning the development of antibiotic resistance in a population. (Events [not in correct order]: Exposure to antibiotics, bacteria population evolution, and random DNA mutations.)

6. As evolution is the change in allele frequency in a population over time …
   a. What does a bacteria need to do to influence the allele frequency in a population?
   b. When considering multi-cellular organisms, what kind of genetic changes will not impact evolution?

Part III – Significance of Evolution Today

"In the antibiotic arms race, it looks like the bacteria are starting to win," I thought. "I wonder what would happen if there really was a super bug that nothing could treat? What if it was a bacterium that caused pneumonia or tuberculosis or cholera instead of just a urinary tract infection?"

In my research, I found that the most important factor leading to antibiotic-resistant bacteria was the presence of antibiotics. I wondered if there was any way to decrease the exposure of bacteria to antibiotics to only when they are needed.

Instructions

When thinking about evolution, many people assume that it is only something that happened millions of years ago and therefore is not important now. So, is evolution relevant today?

To explore this issue, you will discuss in groups the economic, environmental, political, and medical issues associated with antibiotic resistance. Then, as a class, we will discuss the ideas or solutions that you propose for the growing problem of antibiotic resistance.

- Form a group containing one of the following: a journalist, a doctor, a politician, and a parent. The goal of each group is to compile a chart of the many factors that could influence antibiotic resistance.
- If you are a doctor or politician or parent:
    ○ Individually examine the graph at the top of your page and look at the axes. What information does the graph contain? Use this information to write a few sentences about your conclusions.
- If you are the journalist:
    ○ See the chart example to understand how each fact that will be presented by the doctor, politician, or parent may be composed of medical, political, economic, and environmental factors.
- Work as a group to fill out the chart.
    ○ Take turns reading your conclusions from your graph and the facts at the bottom of the page.
    ○ Decide as a group which factors (medical, political, economic, and environmental) each conclusion represents.
    ○ Have the journalist write that information on the chart.
- After the chart has been completed, discuss as a group the question: *How should we reduce antibiotic resistance?*
- Pick a member of your group to present results of your discussion to the class.

Chart Example (especially for journalist)

Example Fact: Traces of prescription drugs like antibiotics are being found in the environment, especially in water. The prescription drugs that are disposed by flushing down the toilet or throwing away in the trash are contributing to this problem.

1. Identify the medical, political, economic, and environmental factors associated with each fact presented by the doctor, the politician, or the parent.
    ○ The above example can impact or be impacted by many of the factors mentioned above. For example, the law could be changed about how we dispose of medicines, but the enforcement and/or education of a new law would cost the government money, thus impacting the economy. The disposal of medicines into the sewer system could impact the populations of bacteria that digest the sewage and these antibiotic-resistant bacteria could be released into the environment. Thus on the chart, the following could be written.
        – Political: A new law would be needed to change antibiotic prescription disposal.
        – Environmental: Medicine disposal could increase environmental bacterial antibiotic resistance.
        – Economic: A new law or educational program would cost the government money.

2. Use the chart on the next page to list the various factors associated with each fact. Note that each fact can involve multiple factors. Below is a sample chart filled in with the factors related to the example fact above.

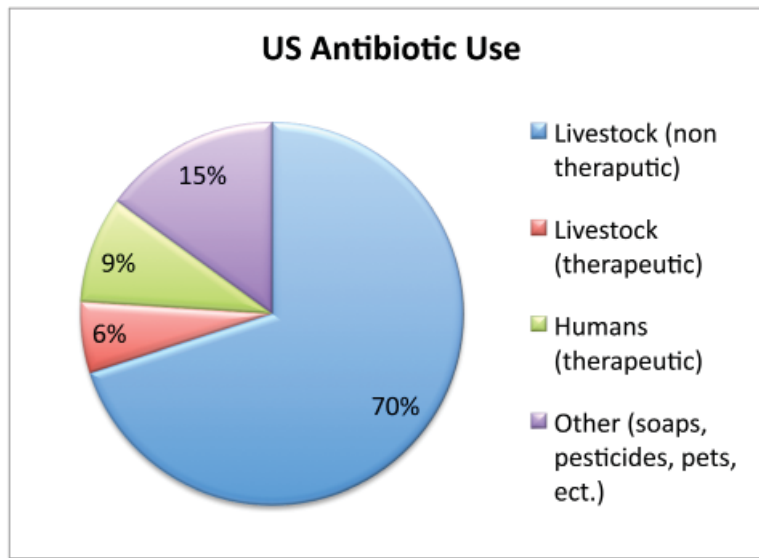| Medical	Political	Economical	Environmental
	A law would be needed to change antibiotic prescription disposal.	A new law or educational program would cost the government money.	Antibiotics flushed down the toilet or thrown in the garbage increase environmental antibiotic resistance.

Factors Associated with Antibiotic Resistance

Medical	Political	Economical	Environmental

Part III – Doctor
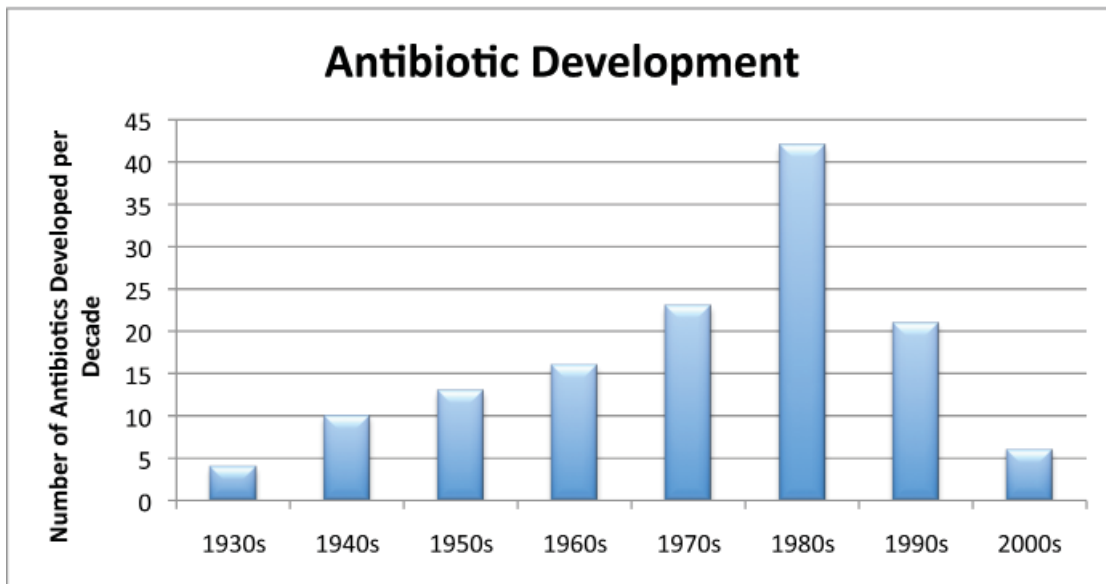
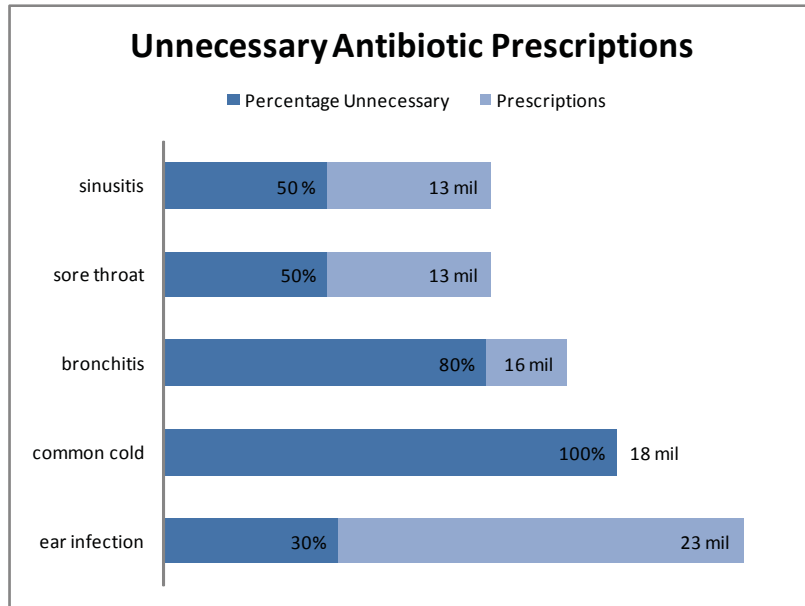

Graph Conclusions:

Consider

- More than 70% of the bacteria that cause hospital-acquired infections are resistant to at least one of the drugs most commonly used to treat them.
- People infected with antibiotic-resistant organisms are more likely to have longer and more expensive hospital stays, increasing the burden on the healthcare system.
- New antibiotics are not profitable to develop. It takes around 500 million dollars and 12-15 years to develop and get a new antibiotic approved. Once the antibiotic is being used, resistance generally develops in 1-5 years.

Part III – Politician

US Antibiotic Use

15%

9%

6%

70%

- Livestock (non theraputic)
- Livestock (therapeutic)
- Humans (therapeutic)
- Other (soaps, pesticides, pets, ect.)

Graph Conclusions:

Consider

- In 1950, scientists discovered that low doses of antibiotics make chickens grow faster than normal. Your constituents are heavily into agriculture and their livelihoods depend upon high yield.
- Reduced antibiotic use may lead to decreased productivity and increased food prices.
- Treating animals with antibiotics allows more animals to be raised on smaller amounts of land than animals not treated with antibiotics. Environmental lobbyists argue that this treatment is inhumane and leads to greater antibiotic concentrations in the environment.

Part III – Parent

Unnecessary Antibiotic Prescriptions

■ Percentage Unnecessary   ■ Prescriptions

sinusitis	50%	13 mil
sore throat	50%	13 mil
bronchitis	80%	16 mil
common cold	100%	18 mil
ear infection	30%	23 mil

More than 50 million unnecessary antibiotic prescriptions are written each year for patients outside of hospitals, according to estimates by the Centers for Disease Control and Prevention. From "Miracle Drugs vs. Superbugs" by Tamar Nordenberg, *FDA Consumer* Nov, 1998.

Graph Conclusions:

Consider

- Over 50% of patients don't finish their prescriptions. This allows partially resistant bacteria to live, so that next time the antibiotic does not kill the bacteria as effectively.
- Body soaps, household cleaners, sponges, and even mattresses and lip-glosses are now packing bacteria-killing ingredients. These products can cause increased antibiotic resistance. Also, these products alter a person's microflora (normal bacteria that exist on skin and in digestive tract).
- Parents and patients often demand antibiotics for sickness even if it is unlikely to help. Antibiotics would be useless for infections caused by viruses for example. But if a doctor does not prescribe the antibiotic, the patient/parent can go to a different doctor. There are currently no laws in the United States regulating how doctors prescribe antibiotics.

Part IV – Checking Your Understanding

It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

Exercise 1

How does antibiotic resistance of bacteria demonstrate various principles of evolution? Make sure to use and underline the following terms in context: *selection, mutation, adaptation, bottleneck, fitness,* and *allele*. Limit your responses to one short paragraph.

Exercise 2

Where do antibiotics come from? What does their source have to do with evolution? Limit your responses to one or two paragraphs.

Exercise 3

Write one page on the role of population diversity in evolution. Be sure to address the following questions:

- What is population diversity? (Differentiate between phenotype and genotype.)
- Why is genetic diversity critical for evolution?
- What would happen to a population or a species if it did not have genetic diversity?
- In detail, how does a population become diverse? (Hint: Explain how mutations occur.)

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Image in the case title block is a colorized 2005 scanning electron micrograph (SEM) depicting numerous clumps of methicillin-resistant *Staphylococcus aureus* bacteria, commonly referred to by the acronym, MRSA. Magnified 2390x. Courtesy of CDC/ Jeff Hageman, M.H.S. This public domain image is ID#10047 and available from http://phil.cdc.gov/phil/details.asp.