Biology Hour\_\_\_\_ Name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  
Wexler/Fennelly  
Lab: Antibiotic Resistant Bacteria in the Environment

**Part I. Prelab**

Article: How do antibiotic-resistant bacteria get into the environment?

September 21, 2016  
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http://theconversation.com/how-do-antibiotic-resistant-bacteria-get-into-the-environment-63856

Antibiotic resistance is a growing public health problem. The United Nations recently acknowledged this as [“one of the biggest threats to modern medicine,”](https://www.theguardian.com/society/2016/sep/20/un-declaration-antibiotic-drug-resistance) dedicating a high-level meeting to the issue at the 2016 General Assembly.

*Why is the U.N. interested in antibiotic resistance of bacteria?*

In the U.S. at least [two million people](https://www.cdc.gov/drugresistance/) are infected with antibiotic resistant bacteria and 23,000 die each year. This problem is largely due to misuse of antibiotics.

*If the U.S. population is 350 million, what percentage of the population  
is infected with antibiotic resistant bacteria each year? (show calculations)*

*If the student population at RUHS is 1600, how many students do we predict will be infected with these bacteria each year? (show calculations)*

Research on antibiotic resistance tends to focus on infection control in hospitals and development of new drugs. But antibiotic bacteria aren’t just found in hospitals or clinics. They – and the genes that confer resistance – are found in the environment, too.

People carry all kinds of bacteria, potentially even resistant bacteria, in and on their bodies. People can shed these bacteria in communal spaces such as locker rooms or [even beaches](http://dx.doi.org/10.1186/1471-2180-11-5), but a major concern is their presence in human sewage. Resistant bacteria enter our aging sewer infrastructure and may eventually end up in the environment through sewage spills. This can expose people to hard-to-treat infections, and creates the potential for genes conferring resistance to be spread to other bacteria in environmental habitats.

*At what locations in our school are you most likely to be exposed to antibiotic resistant bacteria?*

Sampling water and sediment near a sewer line break

In Florida, hurricanes and tropical storms mean that sewage discharges, septic system failures and sewer line breaks happen frequently during the rainy season.

That’s what happened in Pinellas Park, Florida in September 2014. A sewage pipe broke near a school, forcing cars to detour around a parking lot flooded with thousands of gallons of raw sewage. One of the causes was probably was the [3.3 inches of rain fell](http://www.tbo.com/pinellas-county/st-pete-sewer-break-in-front-of-northside-christian-school-20140929/) in the area the weekend before the pipe broke.

[[](https://cdn.theconversation.com/files/138679/area14mp/image-20160921-21701-1490uch.JPG)](https://cdn.theconversation.com/files/138679/area14mp/image-20160921-21701-1490uch.JPG)

The sampling site. Suzanne Young, Author provided

We sampled water and sediment from the site of the initial line break for seven weeks following the spill event, testing samples for VRE (Vancomycin-resistant enterococci).

Finding resistant bacteria and resistance genes in sewage

Vancomycin resistant enterococci have very rarely been detected in surface water or sewage in the U.S., so we weren’t expecting to find these bacteria in the water and sediment from the drainage ditch at the site of the spill. But [we did](http://dx.doi.org/10.1128/AEM.01927-16), as well as thousands of garden-variety enterococci per liter of water. VRE grown in culture were detected up to three days after the date of the spill.

VRE are considered a serious health threat [by the CDC](http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf#page=67), infecting 20,000 people and killing over 1,000 per year. As VRE become increasingly prevalent in hospital settings, it is inevitable that some individuals carry a remnant VRE population when they leave the hospital. These VRE are essentially part of the “normal flora” of these people, and can be passed to others. And people in good health can be colonized by these bacteria without any symptoms. In this way, VRE can spread within the community. Although miles from any major hospital, the “community sewage” in this sewer line may have been collecting the waste of people recently treated with vancomycin or those colonized with VRE.

*Explain why infections by antibiotic resistant bacteria are an increasing problem for patients in hospitals. How can hospitals fix the problem?*

*Bonus Concept: Transfer of Antibiotic Resistance Between Bacteria Species = Lateral Gene Transfer*

One of the most interesting, and worrying, facts about antibiotic resistance is that resistance genes, like *vanA*, are often mobile. These genes can be carried on mobile genetic “cassettes” called transposons. These transposons frequently contain multiple antibiotic resistance genes and can be shared from one bacterium to another (called horizontal gene transfer). *vanA* genes are known to be carried on transposons that can be easily transferred, which are strongly associated with [the spread of VRE infections in hospitals](http://dx.doi.org/10.1086/321815).

Outside of the hospital, a major concern is that other harmful bacteria or even innocuous environmental microbes may acquire these genes when exposed to raw sewage that contains them. The more abundant these genes are in the environment, the more likely they are to be transferred to other, potentially harmful bacteria.

*Almost half the human genome consists of remnant transposable elements, although a small proportion are still quite active and appear to play a role in regulating gene expression. Explain how the presence of these foreign DNA elements is important for human evolution:*

**Part II. Objectives and Procedure**

Your objective is to examine the school environment for ampicillin-resistant bacteria. Ampicillin is a type of penicillin.

Procedure:

Your team is given two agar plates – one has antibiotic, the other does not.

1. Use a marker to divide the plate into six quadrants (mark on the outside of the bottom of the plate).
2. Label the quadrants A-F. Be sure to also label the plates with your hour and team number.
3. Decide with your teammates what environmental samples you wish to examine. Some ideas: your finger, saliva, potting soil, the bottom of your shoe, aquarium water, the handle of a drinking fountain in the hallway, the seat of a toilet, etc.
4. List each sample below:

A)

B)

C)

D)

E)

F)

1. Lightly moisten a cotton swab with distilled water (squeeze out any excess water). Collect sample A using the swab. Spread the sample in a single streak in the A quadrants of both agar plates. Discard the swab.
2. Repeat for samples B-F
3. Keep level and right side up until any moisture on the agar surface has been absorbed.
4. Place upside down in the class bin. These will be incubated at 37°C overnight.

**Part III. Results and Conclusions**

1. In the space below describe your results for each of your six samples. Describe both quantitatively (amount) and qualitatively (looks).  
   A)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

B)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

C)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

D)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

E)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

F)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. Make a concluding statement to summarize your findings and what they mean. Attach a separate piece of paper if you need more room to write.