Determining the Presence of Antibiotic Resistance Genes at the Huston-Brumbaugh Nature Center

Featured scientist: Chessie Misja, University of Mount Union, Collaborating scientist: Dr. Kim Risley

Research Background:
Antibiotic resistance is one of the most pressing public health challenges of the 21st century. As bacteria evolve to survive exposure to antibiotics, the drugs we rely on to treat infections become less effective. This resistance can spread not only in hospitals and communities but also in the environment, especially in soil, which acts as a reservoir for antibiotic resistance genes (ARGs).

How do ARGs end up in soil?
Antibiotics are widely used in agriculture to promote growth and prevent disease in livestock. When animals excrete waste, it often contains unmetabolized antibiotics and resistant bacteria. This waste can be used as fertilizer or can run off into nearby land and water systems. Similarly, human waste from wastewater treatment plants and landfills can introduce antibiotics and resistant microbes into the environment. Over time, these substances accumulate in the soil, where bacteria can exchange ARGs through horizontal gene transfer.

Why study ARGs in soil?
Soil is home to a vast and diverse microbial community. When ARGs are present, they can be taken up by other bacteria, including potential human pathogens. Studying ARGs in soil helps scientists understand how resistance spreads and where it might pose the greatest risk.

In this study, Chessie investigated the presence of three types of antibiotic resistance genes in soil samples from the Huston-Brumbaugh Nature Center (HBNC), a location with both high-traffic and relatively undisturbed areas. The genes Chessie tested for were:

- **ermB (Erythromycin resistance):** This gene provides resistance to macrolide antibiotics like erythromycin by methylating the bacterial ribosome, preventing the antibiotic from binding and halting protein synthesis.
- **Sulfonamide resistance genes (AEW9, SEW2, SEW5):** These genes encode enzymes that bypass the action of sulfonamide antibiotics, which normally inhibit folic acid synthesis in bacteria. The genes target the enzyme dihydropteroate synthase (DHPS), allowing bacteria to continue producing folate even in the presence of the drug.
- **Tetracycline resistance genes (AEG3, SEG6):** These genes typically encode efflux pumps or ribosomal protection proteins that prevent tetracycline from interfering with bacterial protein synthesis. Tetracycline is a broad-spectrum antibiotic, and resistance to it is widespread in both clinical and environmental settings.

By comparing soil samples from areas with different levels of human and animal activity, Chessie aimed to determine whether these interactions influence the presence and distribution of ARGs in the environment.

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In this study, Chessie was interested in exploring if antibiotic resistance genes present in soil samples from different locations at HBNC and if the presence of these genes vary based on human and animal activity in the area.

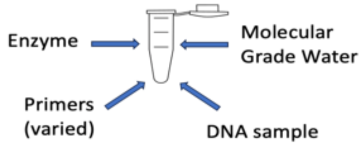
To answer these questions, Chessie completed the following procedures:

Methods

1. 60 total soil samples at 2cm deep were taken, 15 were gathered at 4 different locations at the Huston-Brumbaugh Nature Center (Figure 1).

2. Microbial DNA was isolated and purified from each soil sample using Qiagen DNeasy Powersoil DNA Kit. The 16sRNA gene was amplified to confirm the presence of bacterial DNA in all soil samples.

3. Six different antibiotic resistance genes (primers) were selected and amplified using polymerase chain reaction (PCR) with each pooled sample. Each tube contained 3-5 soil samples. Refer to reference 1 and 2 for specific primer sequences used.



4. Each sample was screened and visualized with gel electrophoresis.

Description of four sites tested

Site 1: Huston Barnyard

Description: This area is near the farm center and barnyard, where human and animal activity are frequent.

Why important: High interaction with livestock and visitors likely increases exposure to antibiotic residues and resistant bacteria.

Site 2: Brumbaugh Woods

Description: A wooded area with older forest growth, less disturbed than the barnyard but still accessible to visitors.

Why important: Represents a semi-natural environment with moderate human activity.

Site 3: Brumbaugh Woods Trail

Description: A trail close to the parking area, suggesting frequent foot traffic.

Why important: High visitor activity could introduce ARGs through soil contact and runoff.

Site 4: Big Valley Trail

Description: A more remote trail with minimal human and animal interaction.

Why important: Serves as a control-like site to compare ARG presence in less disturbed soil

Guiding Questions for Students

Background & Context

1. Why is antibiotic resistance considered a global health threat?

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2. How do antibiotic resistance genes (ARGs) end up in soil environments?

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3. What are the three types of antibiotic resistance genes Chessie tested in this study, and how do they help bacteria survive?

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Research Questions & Hypothesis

5. What was the main research question Chessie was asking in this study?

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6. Propose a null hypothesis for Chessie's study and an alternative hypothesis.

   $H_{(o)}$ ____

   ____

   $H_{(1)}$ ____

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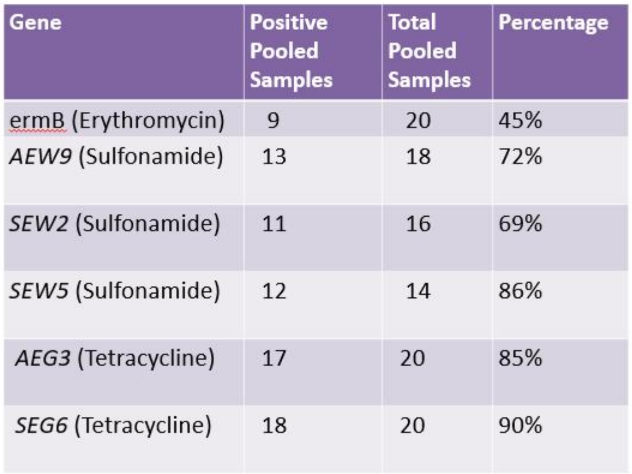
Data & Analysis:

Figure 1. Trail map of the Huston-Brumbaugh Nature Center with specific locations highlighted. 1=Huston Barnyard, 2=Brumbaugh Woods, near Old Forest, 3= The Brumbaugh Woods Trail, near parking lot, 4=The Big Valley Trail



Gene	Positive Pooled Samples	Total Pooled Samples	Percentage
ermB (Erythromycin)	9	20	45%
AEW9 (Sulfonamide)	13	18	72%
SEW2 (Sulfonamide)	11	16	69%
SEW5 (Sulfonamide)	12	14	86%
AEG3 (Tetracycline)	17	20	85%
SEG6 (Tetracycline)	18	20	90%

Table 1. Summary of positive pooled samples.

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Sulfonamide Antibiotic Resistance Genes

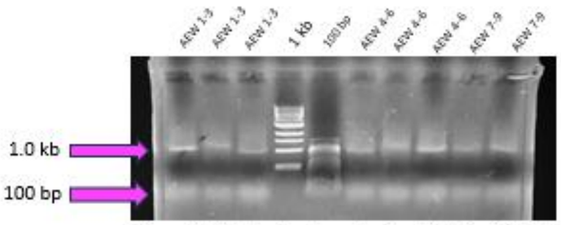

Figure 2. Gel electrophoresis visualization of the *AEW9* gene in soil samples 1-9.

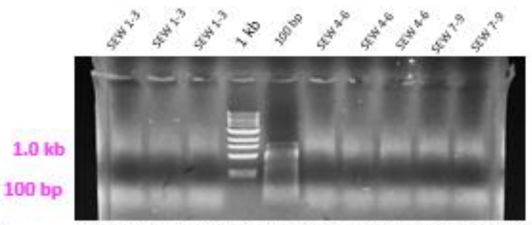

Figure 3. Gel electrophoresis visualization of the *SEW2* gene in soil samples 1-9.

Tetracycline Antibiotic Resistance Genes

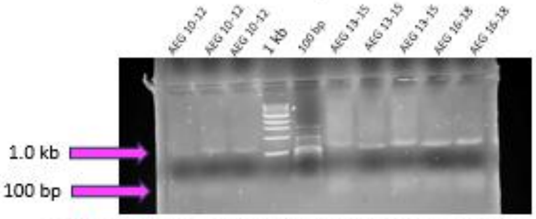

Figure 4. . Gel electrophoresis visualization of the *AEG3* gene in soil samples 10-18.

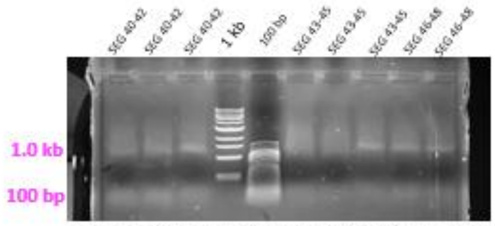

Figure 5. Gel electrophoresis visualization of the *SEG6* gene in soil samples 40-48

Erythromycin Antibiotic Resistance Genes

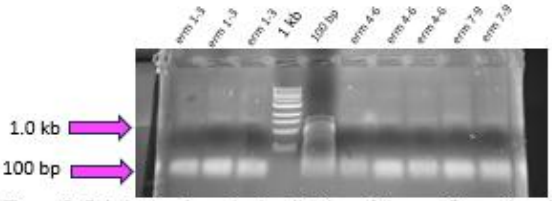

Figure 6. Gel electrophoresis visualization of the *ermB* gene in soil samples 1-9.

Chessie tested soil from four locations at the Huston-Brumbaugh Nature Center to see if antibiotic resistance genes (ARGs) were present. Here's a summary of what she found:

Antibiotic Resistant Genes	Description
Sulfonamide genes (AEW9, SEW2, SEW5)	• These genes were found in most samples except at the Big Valley Trail. • SEW5 was the most common, showing up in **86%** of pooled samples.
Tetracycline genes (AEG3, SEG6)	• These were the most widespread, appearing in almost all locations. • AEG3 was found in **85%** of samples, and SEG6 in **90%**.
Erythromycin gene (ermB)	• This gene was less common, found in **45%** of samples. Most positives came from the Huston Barnyard and Old Forest areas. • The Big Valley Trail and the trail near the parking lot had little to no ermB.

Important Note:

These genes might help microbes survive in their environment, not just resist antibiotics. More research is needed to confirm if these genes make bacteria resistant.

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Student Claim, Evidence, Reasoning Questions:

7. Based on the data, what claim can you make about ARGs at the Huston-Brumbaugh Nature Center? ______________________________________

8. What evidence supports your claim?

9. How does this evidence connect to the hypothesis? Does it support or refute it?

Conclusion:

In conclusion, Chessie determined antibiotic-resistance genes are present at the HBNC. She found most genes were abundant in the areas where there were more human and animal interactions such as the Huston Barnyard and The Brumbaugh Trail, however, it is also important to note that the microbe's genes may be for protection in their environment. The Big Valley Trail only tested positive for two of the genes, indicating that there is not much interaction occurring.

Chessie and her team believe further research is needed to determine if the samples collected are resistant to the antibiotics tested. Future experiments should be designed to complete the following:

1. Positive samples would be cloned into PCR4-TOPO to make a metagenomic library.
2. Screen samples for antibiotic resistance and isolate plasmids.
3. Determine minimum inhibitory concentration (MIC) for 96-well plate or disk diffusion.

Broader Implications

10. Why is it important to monitor ARGs in natural environments?

Citation:

1. **Misja, C. (2023).** *Determining the Presence of Antibiotic Resistance Genes at the Huston-Brumbaugh Nature Center*. University of Mount Union, Alliance, OH 44601

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