BioGraphI

**Biologists and Graph**
**Interpretation Network**

---

# Antibiotic Resistance of Bacterial Soil Isolates and Biofilm Production

A BioGraphI Module Lesson Guide for Instructors

**Original Authors**
Stephanie L Mathews, Assistant Teaching Professor, Department of Biological Sciences, North Carolina State University

Danielle Graham, Associate Professor of Microbiology, Department of Biological and Forensic Sciences, Fayetteville State University

**Course Information**
*Department/Program:* Microbiology Program
*Level*: 300-level, introductory microbiology
*Course type:* Lecture
*Delivery mode:* Online, asynchronous
*Students:* Mixed Majors (microbiology, nutrition, human biology..)
*Number of students:* 300
*Estimated duration of activity:* 60 minutes

**Date of implementation**
November 27, 2023

**Purpose/Background**
This module asks students to interpret data about antibiotic resistance research. It was designed to be used to reinforce learning after asynchronous or in-class lectures on this topic. In addition to applying knowledge about this concept, students will also practice their scientific skills by participating in data interpretation of Kirby Bauer assays as well as a Crystal Violet Biofilm assay. At the end of this module students will also learn about the scientist to build on their understanding of the people and process of scientific discovery.

**List of materials needed for the lesson**

- Slide deck
- There are 4 videos.
- The transcript can be found at the end of this document as well as in a separate document
- Quiz questions are included in the powerpoint file but can also be found in this document if you want to add them to your classroom management system or in-class polling tools. The second page contains the answer key with responses for immediate feedback if your system allows for this type of input.
- Formative and Summative assessment questions are grouped in the same document.
- The prompt for a reflection assignment after this module can be found here.

# About BioGraphI modules

This lesson is a BioGraphI module. BioGraphI modules address data literacy while fostering diversity in undergraduate biology classrooms. They are lessons about graph and data interpretation, featuring the scientific contributions of biologists who are members of historically excluded groups (HEGs). They include video interviews with these biologists, allowing students to hear directly from HEGs about their discoveries. For more information about how the BioGraphI project is advancing inclusion in biology and improving data literacy, visit our webpage.

# Student Learning Objectives

The BioGraphI Student Learning Outcomes (LOs) describe what students can expect to gain by the end of the BioGraphI lesson. They are written in a format that can be shared directly with students.

**Content learning objectives**

1. Predict the effect of antibiotics and immunity on bacterial growth.
2. Explain how improper antibiotic use can lead to an increase in resistance in a bacterial population
3. Apply microbiological knowledge to the analysis of the current of antibiotic resistance.

**Quantitative learning objectives**

4. Interpret graphs and/or data figures related to the concepts from this lesson
5. Reflect on your perceptions about using graphs or figures in biology.

**Diversity/equity/inclusion learning objectives**

6. Reflect on your perceptions of people who do biology.
7. Compare your own interests and/or identities to those of people who do biology.

Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

# Assessments

To help the BioGraphI Project to measure the effectiveness of our modules in improving data literacy and fostering diversity in biology classrooms, we invite your students to participate in a voluntary, anonymous pre-/post-lesson survey (Geneseo IRB #202021048). This survey is designed as an opportunity for reflecting on the Quantitative and D/E/I learning objectives above and administered via LimeSurvey. Click Instructions for access to BioGraphI PrePost-Lesson Student Survey to request a survey to be set up for your students, at least 7 to 10 days in advance of your class meeting date.

| **Objective(s)** | **Formative Assessment** | **Summative Assessments** |
|---|---|---|
| LO1) Predict the effect of antibiotics and immunity on bacterial growth. | Questions interpreting kirby bauer assay.<br>Background questions | Exam MC question: Predict the effective treatment based on Kirby Bauer assay. |
| LO2) Explain how improper antibiotic use can lead to an increase in resistance in a bacterial population | Pre-module Think/Pair/Share: Explain the connection between antibiotic use and antibiotic resistance in a population. | Post-module Think/Pair/Share: Explain the connection between antibiotic use and antibiotic resistance in a population.<br><br>Exam Essay question: explain possible events that led to antibiotic (MDR) resistance documented in MRSA |
| LO3) Apply microbiological knowledge to the analysis of the current of antibiotic resistance. | Reflection forum prompt: find a news article about antibiotic resistance, identify the microbe, antibiotic and mechanism of resistance (if stated).<br>Post in Flip grid. Respond to two peer posts. | Exam Essay question: explain possible events that led to antibiotic (MDR) resistance documented in MRSA |
| LO4) Interpret graphs and/or data figures related to the concepts from this lesson | BioGraphI Student Pre-Lesson Survey (link for instructions to access survey)<br>Embedded video questions | BioGraphI Student Post-Lesson Survey (link for instructions to access survey)<br><br>Quiz question: interpret related graph (same topic, similar methods /measurement) |

Template created by Suann Yang and Rachel Pigg (2022)
Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

| **Objective(s)** | **Formative Assessment** | **Summative Assessments** |
|---|---|---|
| LO5) Reflect on your perceptions about using graphs or figures in biology. | BioGraphI Student Pre-Lesson Survey | BioGraphI Student Post-Lesson Survey |
| LO6) Reflect on your perceptions of people who do biology. | BioGraphI Student Pre-Lesson Survey | BioGraphI Student Post-Lesson Survey |
| LO7) Compare your own interests and/or identities to those of people who do biology. | BioGraphI Student Pre-Lesson Survey | BioGraphI Student Post-Lesson Survey |

# Lesson context

## Learning goals of unit

Two concepts  as identified in the ASM Recommended Curriculum Guidelines for Undergraduate Microbiology Education are:

- Human impact on the environment influences the evolution of microorganisms (e.g., emerging diseases and the selection of antibiotic resistance).
- Bacteria have unique cell structures that can be targets for antibiotics, immunity and phage infection.

A competency  identified in the ASM Recommended Curriculum Guidelines for Undergraduate Microbiology Education is the ability to apply the process of science.

This module emphasizes the concepts and competencies outlined above in the context of a general microbiology course. Students are able to apply their knowledge of microbial physiology to predict if the isolates presented in this research are resistant or susceptible to antibiotics. Students then interpret a graph of biofilm formation of the same bacterial isolates from soil samples. Students may consider the connection between antibiotic resistance and biofilm formation. The results bring to light the importance of the scientific process and how hypotheses can be modified with results for future research.

Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

This module will be assigned for students to complete after viewing lectures on Antimicrobial resistance which address these additional learning objectives:

- Explain how not completing a full treatment of antibiotics can lead to an increase in resistance in a bacterial population.
- Apply microbiological knowledge to the analysis of current social questions or problems.

## Prerequisite skills or knowledge

This module is designed for use at the end of a basic microbiology course. Students should be familiar with the following microbiology concepts:

- **Bacterial Structure:** as students think about antimicrobial resistance, it is important for them to be able to identify structural differences between prokaryotes (in particular bacteria) and eukaryotes.
- **Bacterial Growth**: Students will see a Kirby Bauer assay that depicts bacterial growth on an agar plate. It is assumed that students understand that bacterial growth can be visualized on solid media.
- **Antibiotic mechanisms of action**: Students do not need a comprehensive knowledge of this topic or to have memorized common antibiotics and their mechanism of action, however students should be able to identify that antibiotics prevent growth of bacteria by one of the five major antimicrobial targets (cell wall, cell membrane, nucleotide synthesis, protein synthesis, metabolism) because of structures/processes that are unique to bacteria.
- **Mechanisms of antibiotic resistance**: Similar to the previous topic, students should be able to identify mechanisms of bacterial resistance but do not need to identify specific mutations necessary. It is important that students understand that resistance is due to a genetic change either by random mutation of the target of antibiotics or by horizontal gene transfer. It is a common misconception that bacteria choose to be resistant, or that humans become resistant to antibiotics. While it is true that humans are resistant to antibiotics (if the reverse were true these medications would kill us), bacterial resistance is a process of random mutation and selection pressure.

## Preparation for lesson

- Assign BioGraphI Student Pre-Lesson Survey and a Pre-Lesson Reflection as homework for students to complete before this activity.
- For a basic introduction to using antibiotics to control microbial growth and antimicrobial drugs you may consider having the students read the [Microbiology Open Stax Textbook]() Chapter 14. For more targeted reading have students focus
  - Section 3: Mechanisms of antibacterial drugs
  - Section 5: Drug Resistance
  - Section 6: Testing the effectiveness of Antimicrobials

Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

# Lesson sequence

Each row of this table is a step of the activity. Column headings reveal what the instructor, interviewee, and students do at each step.

To begin, students will take a pre-lesson survey, it is recommended that this survey be emailed to the students a few days before implementing the lesson. In class or in an online learning environment, students will be asked questions about antibiotic resistance. The interview videos are the next components. These videos are split into 4 shorter segments. In between these videos there are formative assessments. In the fourth segment of the video, the instructor or students should pick three of the seven options in which the researcher answers questions about her background, path to be a scientist, and daily life as a scientist. A reflection prompt can be used after the videos as well as a post-lesson survey.

| **Information from instructor (online)** | **Information from scientist (within pre-recorded video interview)** | **Student follow-up or transition activity** |
|---|---|---|
| Prior to class, assign any preparation activities | N/A | Students complete pre-lesson survey for BioGraphI project (combination of reflective writing prompts and closed-ended questions)<br>● *Purpose*: prepare for class; LO 4 & 5 – Pre-lesson reflection on data literacy and perceptions of scientists |
| Introductory Information | | ● Think/Pair/Share activity about antibiotic resistance<br>● Student interpret Kirby Bauer Assay; LO1 |
| Plays first video segment 9:20 | ***Hypothesis***<br>***Methods***<br>***Qualitative findings*** | **Fundamental activity:** Students reinterpret Kibry Bauer from research.<br>Evaluate whether or not the hypothesis was supported.<br>● *Purpose*: LO 3 & 4 - Check student understanding of the concepts and vocabulary |
| Second Segment of video 2:50 | ***Researcher explain meaning show quantitative data***<br>***Introduce graph*** | **Fundamental activity:** Students interpret graph, determine measurements and relate to research questions in embedded questions.<br>● *Purpose*: LO 3 & 4 - Check |

Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

| | | |
|---|---|---|
| | | student understanding of the concepts and vocabulary |
| Third Video Segment 11:21 | ***From the Researcher*** Orient us to this graph we've been working on….<br>Tell me about the research that these data came from…..<br>Tell me about the research that these data came from: Explain the significance of this research | Based on these results, what should the scientist investigate next? Share out.<br>● *Purpose*: Align to implications outside of this research LO 2<br>*Purpose*: Closes this portion of the lesson |
| Fourth Video Segment 15:13 | ***From the Researcher***<br>What kinds of scientific questions interest you the most?<br>When and how did you know you wanted to be a scientist?<br>Tell us about the paths that led you to your current job.<br>What is it like being a scientist who identifies as…?<br>Tell me about a moment when you felt like you really belonged in the field of science.<br><br>*These questions are also segmented in video clips for students to choose 3 of these questions* | *Students select three of the seven video segments*<br>● *Purpose*: LO 4 - Identifying potential connections between students and scientist |
| Instructor shares email of scientist so that students can ask leftover questions! | | ● Think/Pair/Share activity about antibiotic resistance<br>Reflection forum prompt:<br>● *Purpose*: LO 4 - Identifying potential connections between students and scientist.<br>● LO3 apply knowledge to current context<br>Students complete post-lesson survey for BioGraphI project<br>● *Purpose*: LO 2-5 – Post-lesson reflection on data literacy and perceptions of scientists |

Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

# Alignment to Universal Design for Learning Guidelines

| UDL Guideline | Lesson Alignment |
|---|---|
| Multiple means of Engagement | **Self-regulation**: Students reflect on their dispositions and knowledge of scientists and graph interpretation before and after the lesson (Embedded MC questions, Pre and Post surveys to reflect on self-efficacy and scientific identity)<br>**Recruiting Interest:** Discussion forum with choice for article and flipgrid to vary delivery/assignment style for students<br>**Sustaining effort**: Immediate feedback built into embedded MC questions |
| Multiple means of Representation | **Perception**: Captions are provided on the interview video. Transcript . Alt text for images provided in Google Slides.<br>**Language and Symbols:** Using qualitative and quantitative data. Symbols defined in figures and tables. Addition of visual cues for soil sample types.<br>**Comprehension:** This is linked to several previous course examples: MegaPlate video and presented Kirby Bauer |
| Multiple means of Action and Expression | **Physical Action:** Use of video, slides, MC, flipgrid, and short answer<br>**Expression & communication:** Multiple types of media are used in the lesson (visual, audio, text). |

# Implementation notes

Overall, students responded positively to this lesson.

- They commented that they liked hearing from a researcher and that it featured undergraduate work.
- Students appreciated that they could pick to listen to videos from the researcher for the fourth video segment.

Template created by Suann Yang and Rachel Pigg (2022)
Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

- Some students commented on the reflection prompt and how they liked researching a pathogen and connecting ideas from this research to a clinical application.

Formative assessment questions were graded for participation such that students can apply their knowledge without a fear of failure. Summative assessment questions demonstrated their understanding of antibiotic resistance.

## Suggestions for adaptations

**Asynchronous:** This lesson was first implemented in an asynchronous class as a learning module in the online course management system. Students navigated to a different page for each component of the lesson and formative assessment questions were embedded into the video rather than presented on slides.

1. Pre-lesson formative assessment
2. Video segment 1 with embedded question
3. Video segment 2 with embedded questions
4. Video segment 3 with embedded questions
5. Page with links to seven videos with instructions to watch 3
6. Post-lesson formative assessment questions
7. Reflection question

In this asynchronous design, students appreciated the interactive quizzes that were embedded in the videos. Some students however did not appreciate the design of the lesson in that it did not allow them to move freely between the pages.

**In person:** The slides provided allow for implementation in in-person course meetings. It is recommended that you use a classroom response system or a structure to allow everyone to respond to the formative questions. It may be that students do not have experience interpreting graphs or in the case of this research, are confused when the outcome doesn't prove the hypothesis. Response systems can anonymize responses and ensure full participation in the activity.

# Acknowledgements

We would like to thank Dr. Ariane Peralta, at East Carolina University, for providing the WRC samples. We would also like to thank Sarina Maybank who performed all of the experiments while an undergraduate student at Fayetteville State University.

Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

# References/resources

1. OpenStax; Parker, Nina; Schneegurt, Mark; Thi Tu, Anh-Hue; Forster, Brian M.; and Lister, Philip, "Microbiology" (2016). *Open Educational Resources Collection*. 3. https://openstax.org/details/books/microbiology

# Funding for BioGraphI

This project is funded by the Directorate for Biological Sciences, Division of Biological Infrastructure as part of efforts to address the challenges posed in Vision and Change in Undergraduate Biology Education: A Call to Action (http://visionandchange/finalreport/). Award number 2120679.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

# Transcript of interview

**SUMMARY KEYWORDS**
biofilm, antibiotic resistance, research, isolates, antibiotics, soil, bacteria, nutrient, different types, students, organisms, scientist, fayetteville state university, biofilm formation, samples, optical density, undergraduate students, resistance, bacterial, measure

**SPEAKERS**
Stephanie Mathews, Danielle Graham

**Stephanie Mathews** 0:00
Hi, Danielle. Thanks for meeting with me today. So today we'll have an interview with Dr. Danielle Graham, who is an associate professor at Fayetteville State University in the Department of Biological and forensic science, and she and I are both microbiologist, but today we're going to talk about her research. Can you tell me what kinds of questions interests you most when it comes to your research?

**Danielle Graham** 0:24
Absolutely. So thank you so much for inviting me for this interview. I'm excited to talk to you about some of the things that are really interesting to me. And so I'm really interested in how antibiotic resistant bacteria evolve, how they persist and what new ways we can develop approaches to explore and fight against antibiotic resistance. A lot of my training is in genetics involving bacteria. And so I'm interested in fundamental genetic and molecular processes and approaches that control bacterial biofilms and we'll talk a little bit later about biofilms as well, and developing strategies to disrupt them and prevent them, especially in clinical contexts. I'm

Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

also interested in finding new antimicrobials we have a lot of antimicrobials and antibiotics that fight against some of the bacteria that are well known. However, we know that those organisms become resistant. And so finding new antimicrobials and how they can work to get rid of or alleviate this, the different types of bacterial pathogens is something that really interesting.

**Stephanie Mathews** 1:35
Yeah, that's exciting. And so one of the reasons that I invited you for this interview is because I'm also really interested in learning more about novel antimicrobials and ways that we can combat the antimicrobial issue. So today, we're going to focus on one of your many projects. So can you tell us what started this project that we're about to learn more about?

**Danielle Graham** 1:58
Absolutely. So in my prior training, I focused a lot on clinically relevant bacterial organisms. And I worked at larger research institutions. However transitioning to Fayetteville State [University]. I wanted to find a research project that was really suitable for undergraduate students. So I use my background that focused on clinically relevant bacterial pathogens, along with a global crowdsourcing initiative, to design a project that really addresses the growing concerns of antibiotic resistance.

**Stephanie Mathews** 2:26
So the work that we're going to hear about today is undergraduate research Right?

**Danielle Graham** 2:31
Right.

**Stephanie Mathews** 2:32
That's awesome. I'm so excited for so many reasons. But when we first chatted about this project, you told me about your research hypothesis and that long term nutrient enrichment will increase the percentage of antibiotic resistance and biofilm formation in wetland soil isolates. Can you explain how you might design a research experiment to look at antibiotic resistance and the amount of resistance that we see with nutrient enrichment and the biofilm production capability related to that?

**Danielle Graham** 3:00
Yeah, absolutely. Um, so we wanted to test our hypothesis that long term nutrient enrichment, increases antibiotic resistance and biofilm formation, particularly in wetland soil isolates. And so what I didn't mention is that a part of developing my project was to reach out to colleagues that also have a similar interest, but to expand the project I've collaborated with a colleague at East Carolina University to develop this project using soil isolate from this nutrient enrichment area. And so we collect soil samples from this wetland area. And then from the soil we isolate bacteria, we culture it in different nutrient media that would be conducive to bacterial growth, from organisms that live in the soil. Once those organisms are isolated, we can then assess antibiotic resistance using some techniques that we'll talk about later, such as Kirby Bauer and just monitoring their resistance levels. Simultaneously, we can also measure the ability of these

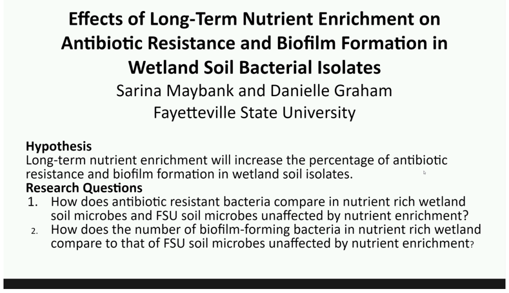
Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

isolated bacteria to form biofilms using the data, we can analyze it to determine if there's any differences between our control group and our nutrient enriched groups, and we can draw conclusions based off those results. That could provide us with further evidence to support or refute the hypothesis. Awesome.

**Stephanie Mathews** 4:26
So you told us where you collected the soil samples in collaboration with a colleague at ECU. But can you tell me a little bit more about soil sample collection?

**Danielle Graham** 4:38
Absolutely. So we wanted to collect a diverse set of samples and so they campus at ECU has a specific research area and this research area was established in 2003. And it was really developed as a resource for undergraduate students. And so this is a wetland habitat that is located a few miles off the campus of ECU and there's blocks of land that is treated annually with fertilizer and those that are not. And so researchers and students can go to these different plots of land, and they can collect soil in order to perform different types of experiments. And in my case, being a microbiologist I'm interested in the soil because it is inhabiting of different types of bacteria organisms. A little bit later when we talk about the data, you'll see that another set of samples was collected from Fayetteville State University's campus. Fayetteville State is located in the southeastern region of North Carolina. And so I really encourage my students to explore that campus and to collect different soil from sites on campus just to intrigue them and to have some type of tie to the university.

**Stephanie Mathews** 6:01
Thanks for telling us about the research site at ECU. It's interesting that there's different nutrient levels and different types of treatment, which we can see in this image here, as well as the differing soil on your campus. Why in particular, did you pick these sites?



**Effects of Long-Term Nutrient Enrichment on Antibiotic Resistance and Biofilm Formation in Wetland Soil Bacterial Isolates**

Sarina Maybank and Danielle Graham
Fayetteville State University

**Hypothesis**
Long-term nutrient enrichment will increase the percentage of antibiotic resistance and biofilm formation in wetland soil isolates.
**Research Questions**
1. How does antibiotic resistant bacteria compare in nutrient rich wetland soil microbes and FSU soil microbes unaffected by nutrient enrichment?
2. How does the number of biofilm-forming bacteria in nutrient rich wetland compare to that of FSU soil microbes unaffected by nutrient enrichment?

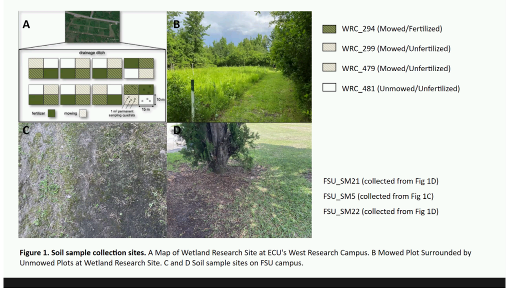

A
drainage ditch
fertilizer
mowing
1 m² permanent sampling quadrats
10 m
15 m
B
C
D
WRC_294 (Mowed/Fertilized)
WRC_299 (Mowed/Unfertilized)
WRC_479 (Mowed/Unfertilized)
WRC_481 (Unmowed/Unfertilized)
FSU_SM21 (collected from Fig 1D)
FSU_SM5 (collected from Fig 1C)
FSU_SM22 (collected from Fig 1D)

**Figure 1. Soil sample collection sites.** A Map of Wetland Research Site at ECU's West Research Campus. B Mowed Plot Surrounded by Unmowed Plots at Wetland Research Site. C and D Soil sample sites on FSU campus.

Template created by Suann Yang and Rachel Pigg (2022)
Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

**Danielle Graham** 6:16
That's a great question. Well, one, thinking back to engaging undergraduate researchers and having available resources, soil is everywhere. So that was a very good selection. Because we can readily assess it not only at ECU, but also like that will state we're gonna walk right out the door and collect samples that we can use back in the lab, but looking a little bit deeper into soil soil is rich in microbial diversity. It plays a crucial role in maintaining different biodiversity and ecosystem functions. However, we know that human activities can result in changes in the soil microbial communities. So we wanted to select soil samples from specific locations such as the site at ECU that we know have, has been used for research purposes and underwent some changes that would then be applicable to different types of human activities and determine whether or not those changes in the soil environment can influence bacterial traits such as resistance and biofilm formation.

**Stephanie Mathews** 7:28
That's awesome. So now that we know more about your materials and where you sampled, let's go back to some of your research questions. You first wanted to measure antibiotic resistance and how exactly did you measure antibiotic resistance in your soil isolates from these interesting sites?

**Danielle Graham** 7:44
That's a great question. So a very common way to measure Antibiotic resistance is by using a Kirby Bauer test is also known as a disc diffusion test. So this is a method in which you would spread a bacterial culture and do you have your isolate over the surface of an agar plate. And once your bacteria is evenly coated across the agar plate you can then place antibiotic discs that have different concentrations of antibiotics on top of the agar. As the bacteria begin to grow, we incubate that agar plate at different temperatures, because we are growing soil isolates. We want to incubate it out of certain temperatures that is going to be inducive for bacterial growth in the soil. So that's usually between 23 and 28 degrees Celsius. During that grow, we can see whether or not the bacteria are one resistant to the antibiotics, meaning that they're going to grow and you will see visible growth around it this or if they're susceptible, meaning that they'll be inhibited and we'll see a zone clearing around this. So depending on the size of the zone of clearing, also referred to as the inhibition zone, this can be measured and then we can compare it to different standardized guidelines. And that's going to determine whether or not that organism is resistant to that particular antibiotic or if it is susceptible.

**Stephanie Mathews** 9:11
That's awesome. And so this technique is used in a clinical setting too, but you're using it in the research setting, as well.

**Danielle Graham** 9:19
Absolutely, yeah.

Template created by Suann Yang and Rachel Pigg (2022)
Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

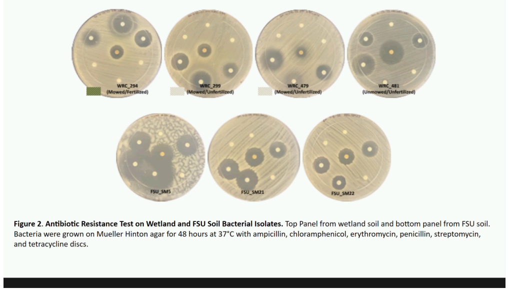


**Figure 2. Antibiotic Resistance Test on Wetland and FSU Soil Bacterial Isolates.** Top Panel from wetland soil and bottom panel from FSU soil. Bacteria were grown on Mueller Hinton agar for 48 hours at 37°C with ampicillin, chloramphenicol, erythromycin, penicillin, streptomycin, and tetracycline discs.

[TRANSITION TO EMBED QUIZ] [TRANSITION TO SECTION 2]

**Stephanie Mathews** 9:25
Alright, so now we can see your exciting results. It's really interesting that just in the seven different soil samples that you took, you were able to find some zones of inhibition. And so you see that the antibiotics are working, but you also see some resistance. When you measure these zones, what exactly did you find?

**Danielle Graham** 9:44
So when we analyze the results, and we measure the diameter of the zone inhibition, we've noticed that all soil samples were resistant to penicillin. And so you may have heard of penicillin is a very common antibiotic. And it was the first antibiotic that was discovered. And so it is no surprise that bacteria have evolved over the many years to be resistant to this particular antibiotic. And so, antibiotics they also have different mechanisms of action on how they function to destroy or inhibit a cell. And so penicillin works in one way. However, some of the other antibiotics such as chloramphenicol inhibits the growth in a different way. However, we saw that the soil samples were susceptible to chloramphenicol, which has a different mechanism of action compared to penicillin. Looking at some of the other antibiotics such as ampicillin, erythromycin, streptomycin and tetracycline, they have varying levels. of resistance susceptibility, and you'll also see intermediate so depending on the zone, or the diameter, we can read it as resistance, sensitive, or intermediate. And those numbers are reflected in that chart. And so the enriched soil meaning that the organisms that came from a plot of land that were enrich or fertilized, they had half of their resistance to antibiotics and this was similar to unfertilized samples. And so we'll talk about comparing these results to the hypothesis as we see right now that we didn't find any major resistance compared to the different types of types of fertilizer.

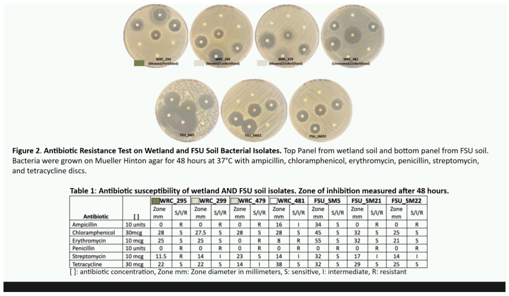


**Figure 2. Antibiotic Resistance Test on Wetland and FSU Soil Bacterial Isolates.** Top Panel from wetland soil and bottom panel from FSU soil. Bacteria were grown on Mueller Hinton agar for 48 hours at 37°C with ampicillin, chloramphenicol, erythromycin, penicillin, streptomycin, and tetracycline discs.

**Table 1: Antibiotic susceptibility of wetland AND FSU soil isolates. Zone of inhibition measured after 48 hours.**

| Antibiotic | [ ] | 🟩 WRC_295 | | ▨ WRC_299 | | ▨ WRC_479 | | ☐ WRC_481 | | FSU_SM5 | | FSU_SM21 | | FSU_SM22 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Zone mm | S/I/R | Zone mm | S/I/R | Zone mm | S/I/R | Zone mm | S/I/R | Zone mm | S/I/R | Zone mm | S/I/R | Zone mm | S/I/R |
| Ampicillin | 10 units | 0 | R | 0 | R | 0 | R | 16 | I | 34 | S | 0 | R | 0 | R |
| Chloramphenicol | 30mcg | 28 | S | 27.5 | S | 28 | S | 28 | S | 45 | S | 32 | S | 25 | S |
| Erythromycin | 10 mcg | 25 | S | 25 | S | 0 | R | 8 | R | 55 | S | 32 | S | 21 | S |
| Penicillin | 10 units | 0 | R | 0 | R | 0 | R | 0 | R | 0 | R | 0 | R | 0 | R |
| Streptomycin | 10 mcg | 11.5 | R | 14 | I | 23 | S | 14 | I | 32 | S | 17 | I | 14 | I |
| Tetracycline | 30 mcg | 22 | S | 22 | S | 14 | I | 38 | S | 32 | S | 29 | S | 25 | S |

[ ]: antibiotic concentration, Zone mm: Zone diameter in millimeters, S: sensitive, I: intermediate, R: resistant

Template created by Suann Yang and Rachel Pigg (2022)
Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

**Stephanie Mathews** 11:41
Yeah, it's really interesting that you're able to take this qualitative data and quantify and determine the levels of resistance in terms of susceptible or intermediate or resistant and that relates back to your first question of looking at how enrichment may or may not play a role in antibiotic resistance. Now we're gonna move to your second research question. And so here we're looking at the number of biofilm forming bacteria and how it compares with the nutrient and rich soil and the wetland and that of the soil sample collected at Fayetteville State University that would be unaffected by nutrient enrichment. And now they're going to try to interpret this graph before you explain it.

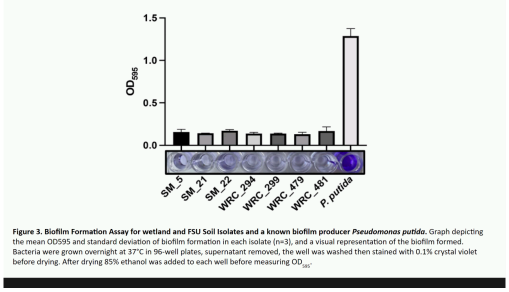


**Figure 3. Biofilm Formation Assay for wetland and FSU Soil Isolates and a known biofilm producer *Pseudomonas putida*.** Graph depicting the mean OD595 and standard deviation of biofilm formation in each isolate (n=3), and a visual representation of the biofilm formed. Bacteria were grown overnight at 37°C in 96-well plates, supernatant removed, the well was washed then stained with 0.1% crystal violet before drying. After drying 85% ethanol was added to each well before measuring $OD_{595}$.

[TRANSITION TO EMBEDDED QUIZ] [TRANSITION TO SECTION 3]

**Stephanie Mathews** 12:13
Dr. Graham, can you orient us to this graph? What do these axes mean?

**Danielle Graham** 12:18
Absolutely. So in a typical graph, the y axis is the vertical line on the left, and it shows that up and down the vertical information is where you'll find data that you're measuring, or what you want to understand. On the y axis. We're measuring the optical density. So this relates to the biofilm formation on the x axis. This is the line that is running horizontal or along the bottom. And it's going to show the information that represents different variables that you're comparing. In this case, we're comparing different soil isolates and we're measuring or comparing them against each other. And so, we're looking to see and these various soil isolates that we collected from either our nutrient rich fertilized plots of land or from different soils collected on campus, can produce biofilm formation, and we measured that by measuring the optical density.

**Stephanie Mathews** 13:30
Thanks for orienting us to the graph and explaining how you measure what we're looking at which is biofilm formation. One thing that I want to ask is we have a new sample here, P Putida. Can you tell us what that is and what it means?

**Danielle Graham** 13:40

Template created by Suann Yang and Rachel Pigg (2022)
Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

Absolutely. So, along with your experimental group, you often want to include controls. And so a control can either serve as a positive control meaning that you expect to see a result or a negative result. Really don't expect to see a result. But this will let you know that your experiment at least is working properly. So P putida. is abbreviated and that P stands for Pseudomonas. And so this is a bacterial organism that we know can produce biofilm. And so that was included in this particular chart and graph so that we have a positive control compared to our experimental samples.

**Stephanie Mathews** 14:25
So how did you make this particular graph and why did you decide to show the data in this way?

**Danielle Graham** 14:30
So to measure biofilm formation, we created a bar graph. We use a particular software called PRISM. I really liked that software because it's pretty easy to use. You can enter in your data is similar to Excel where you entering your data. It will graph the x and y axis, but you can also analyze your data and generate different types of statistical texts. And so you placed the names in your bacterial samples along that horizontal line and those serve as the independent variable and the biofilm. Data is then measured and this is measured based on optical density. Optical density is then graphed here across this line that runs up and down. And so that is going to show you how much biofilm was formed in each particular sample. So underneath this graph, I've also included a visual and this is just one row of the wells, and you can see based off this color here, as it intensifies, you have a larger bar graph and that's indicative to how much the OD is measuring. And this also correlates to how much biofilm is there.

**Stephanie Mathews** 15:50
If you're a microbiology student, you're probably pretty familiar with this color. Can you tell us why we're seeing purple?

**Danielle Graham** 15:58
So the reason why you are seeing purple there is because we're using a particular stain called Crystal Violet. And so crystal violet is a common stain that can use to adhere to different bacterial organisms in this particular case, we're using it to adhere to biofilm. What I didn't mention is that the bacteria are grown in microtiter plates, 96 Well, microtiter plates, and they're allowed to grow in that plate, over many hours, roughly about 18 hours. And so the bacteria they adhere to the plastic, it is a plastic plate, and they here to it and they start to produce biofilm. And after the 18 hours is up, we rinse away in cells that are not attached and then we add that stain which is purple and so in measuring optical density, it measures basically how much is there. So if the color is clear, you're going to get a lower number if the color is more clouded or darker, you're going to get a higher number and so the crystal violet can then bind to biofilm. And the darker the color, the more intense or the higher number of you'll, you'll read in the optical density.

**Stephanie Mathews** 17:30

Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

So you're still using Yeah, so you're still using the same Dye as a gram stain. But in this case, you're just using it to stain the biofilm. And you can quantify the amount of dye instead of like in the Gram stain you're looking at is it purple, or is it pink?

**Stephanie Mathews** 17:23
Can you tell me about the research that produced this data?

**Danielle Graham** 17:27
Absolutely. So we were really interested in determining whether long term nutrient enrichment would increase antibiotic resistance and biofilm formation in wetland soil isolates. And so this project was in combination with just studying changes in different types of bacterial organisms that are exposed to nutrient enrichment over time. So based off our results, we determined that wetlands low isolates contain a higher percentage of antibiotic resistant bacteria compared to the samples that we isolated from Fayetteville State. But there were no significant difference between the different plots of land that we use to collect samples from ECU. So our original hypothesis was not supported. We wanted to look further into this and determine some of the different variables that resulted in what we found. But this really highlights the complexity of the relationship between nutrient enrichment, antibiotic resistance and also biofilm formation, particularly in wetlands or isolates. So this just suggests to us that we need more research to really fully understand what's going on.

**Stephanie Mathews** 18:50
This is great, right, this is the process of science. We have a hypothesis that we perform our experiments, and then we analyze the results and the results suggest that there's more research to come so can you tell us about what those expensive, what those potential future experiments will be?

[TRANSITION TO EMBEDDED QUIZ]

**Danielle Graham** 19:12
Absolutely. So we are really interested in learning and better understanding how nutrients and nutrient enrichment affect bacterial traits, not only the physical traits, but we want to understand the genetic differences as well. And so we want to compare the genetic code between nutrient enriched bacteria to those that are not and perform whole genome sequencing to see if there aren't any genetic differences between these organisms.

**Stephanie Mathews** 19:39
Yeah, that's really interesting. So you used a lot of physiological characterization in terms of like growth or biofilm production, but next you're going to move to more genomic studies. That should be really rich. That's awesome. So, what do you do if you have questions about how to analyze your data?

**Danielle Graham** 19:56

Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

That is such a great question because even I as a researcher and I've been doing this for many years, always have questions about how best to analyze your data to really understand your results. And so I typically seek advice and support from my colleagues, particularly my university or peers that are in similar STEM fields. As I mentioned earlier, I work with a collaborator at ECU. And so we hold weekly meetings to discuss the data that we're collecting. So we just have a different perspective and we can look at it and determine any differences. Another really important thing that I tried to incorporate throughout the years professional development, such as attending workshops and conferences, and this allows me just to expand my knowledge to network and refine my skills. So these interactions both with my colleagues and also at professional development opportunities, just promote a very dynamic learning environment. It helps foster a supportive environment and it just leads to a more productive scientific research project.

**Stephanie Mathews** 21:13
Yeah, it's so important to collaborate in science. I find that this is true as well. So as we think about what is to come and how we collaborate together, I'm excited to see your future results. But can you help or can you elaborate on the significance of this research?

**Danielle Graham** 21:30
Absolutely. This research directly addresses how nutrient enrichment impacts antibiotic resistance and we know how important Antibiotic resistance is and understanding the differences in bacteria in trying to combat resistant organisms as well as biofilm formation, which is another factor that plays in bacteria being able to avoid treatment and particularly in soil microorganisms. So this carries implications both and just the field of microbiology and the society at large. So the improper use of antibiotics can 1) lead to the rise of resistant bacteria, which is a major global concern. And so this study helps understand the connection between nutrient enriched environments and how different products that we do apply to the soil and affect organisms. And later on, we may consume either agricultural food products from those lands and how that can affect us. 2)Also with biofilm formation, which is another very serious and significant issue, especially in healthcare that we want to understand how biofilm formation is being produced and how we can manage it or prevent it. And so this research is just going to offer new insights and better inform us about antibiotic management and we want to align our research goals, particularly to just start discovering any advances that relate to alleviating biofilm formation and decreasing different types of organisms that do become resistant to antibiotics.

**Stephanie Mathews** 23:16
That is really exciting. And it also gets that that not only can we in terms of how we take antibiotics directly influence the issue of resistance but also indirectly in terms of how we treat soil or other just many other ways that we go about daily life. Maybe not just directly treating a disease.

[TRANSITION TO SECTION 4]

Template created by Suann Yang and Rachel Pigg (2022)
Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

**Stephanie Mathews** 23:36
So now we're going to transition to talk just a little bit about you. How did you know that you wanted to be a scientist?

**Danielle Graham** 23:43
So I realized that I want to be a scientist, probably my freshman year of college. I initially entered college, knowing that I wanted to major in STEM finding a passion for biology. And thinking that I was going to pursue a career as a physician. However, my freshman year I joined a research program, and I really loved being part of that research project. And I started to be mentored by a professor here that was a microbiologist and so I was really fascinated that very tiny organisms that we cannot see with our eyes can cause such significant health diseases. And so this passion led me to want to find different treatments for these diseases and just be a part of this solution. So as a first generation college student, navigating this path to being a scientist was very new and challenging, but I really found inspiration in my mentor, who was also a black female scientist. So she not only shared my ethnicity, but also my aspirations because I could really see myself in her, so seeing how successful she was and how she excelled in her field. reassured me then pretty much I could do it too. And I had her support. So she served as a role model and that combined with the different resources that was provided at my institution, through like a mentor program, it just helped me overcome some of those barriers and made my dream of becoming a scientist realistic.

**Stephanie Mathews** 25:24
That's awesome. Can you tell us about the path that led you to your current job as you are a professor at Fayetteville State University?

**Danielle Graham** 25:30
Absolutely. So I received my bachelor's in Biology from Fayetteville State University so I am alone, the institution. I then went on to earn my PhD in microbiology and immunology at the University of Arkansas for medical sciences. I heavily focused on the microbiology aspect. I do have a little bit of training and immunology as well. So during my doctorate I studied bacterial organism that causes Lyme disease and following my doctorate, I pursued a postdoctoral fellowship. And so this is just further training after receiving a doctorate degree, and I completed that training at University of North Carolina at Chapel Hill. We're also focused on another clinically relevant bacterium called Clostridium difficile, during this phase, so during my postdoc, I also participated in a federally funded program that specifically trained individuals for careers in academia. And so this program helped enhance my teaching. It helped enhance my research. skills. And so after my postdoc, I applied for the position here at Fayetteville State University, and I now serve as a professor. I run my research lab, I have undergraduates that work in my research lab and also serve as an administrator as a department chair in biology in forensic science. So here at Fayetteville State, I can really continue to explore my passion for Microbiology research, my passion for engaging undergraduate students and just contribute to the development of the next generation of scientists through both education and mentorship. Similar to what I received when I was a student here at Fayetteville State.

Template created by Suann Yang and Rachel Pigg (2022)
Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

**Stephanie Mathews** 27:21
So it sounds like in a short while maybe not that short, but maybe retrospectively, it feels short. You went from being probably pre med, to research focused and then to academia. So that's really interesting. Would you say that it was a large part of learning about what's possible by the mentors who surrounded you and then just like finding your passion?

**Danielle Graham** 27:42
It was most definitely a combination of both of those because stepping into research, my freshman year I had no idea what that meant. And so seeing it firsthand, and also participating and externships, so visiting different campuses during the summer, to conduct research on more research intensive universities was really instrumental and just having mentors that was able to provide me with the right guidance over time was really important in shaping my career.

During my my PhD, the organism I used to say Lyme disease because people immediately know what that is, but the organism that causes Lyme disease is really Borrelia Burgdorferi. And so, that name is the scientific name, but it's often referred to as a Lyme disease causing bacteria.

**Stephanie Mathews** 28:39
I feel like if you were going write a bacterial tongue twister, that's fun to say it would be Borrelia burgdorferi or some sort of play on that. Dr. Graham, what is it like being a black female scientist in a STEM field?

**Danielle Graham** 28:52
That's a really good question. So being a black female scientists and STEM is both inspiring and challenging. It's fulfilling to be a role models, especially for those with similar backgrounds. Just to motivate them to pursue science careers. But nothing comes without challenges and hurdles, especially impostor syndrome, and stereotypes, but I am able to overcome these things by the support of my mentors in different programs that have assisted me. And so despite these obstacles, really representation and mentorship in STEM are powerful forces, and so I really enjoy being relatable to my students and just being a source of motivation to let them know that they can also do it as well. And being part of this group is, I really just want them to feel included. And so I think some of the things that I'm doing now is breaking down those barriers of putting labels on ourselves and just being part of the field in general.

**Stephanie Mathews** 30:00
Yeah, that's really awesome that you were encouraged to start as a scientist by being an undergraduate researcher and you're providing that opportunity now. to students. But can you tell me about a time where you felt like you really belonged in the field of science?

**Danielle Graham** 30:14
I thought about this. And I think it's been multiple moments where I had a sense of belonging, but I would definitely say now, more in my role as a mentor, and a role model, inspiring scientists or future scientists, is where I'm really feeling my sense of belonging. So I really enjoy

Template created by Suann Yang and Rachel Pigg (2022)
Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

working with undergraduate students because they're very enthusiastic and determined, they still have a lot of drive and passion for what they are pursuing. And so for me it's just a very good reminder that my personal journey to become a scientist is important to others, and me being able to share my research skills and mentoring, and teach validates that I know how to do science. I know how to set up experiments. And so it's really just a sense of belonging when I'm able to connect with my students and teach them things that's gonna be important for them to be successful as scientists as well.

**Stephanie Mathews** 31:25
Thank you so much for sharing that and for helping us to understand what it looks like to to find belonging in science, which is sometimes hard. What is a typical day like for you?

**Danielle Graham** 31:36
A typical day is very busy. I mentioned before that I wear multiple hats. So I am a professor. So I teach courses. I am a researcher and I run an independent research lab. And I also serve as a administrator as a department chair. So my days are quite busy. I usually begin my day with coffee and addressing emails particularly those related to like faculty and students, and then everything else follows. My course depending on if it's online or if it's in person, I like to check in with my students and make sure I don't have any outstanding tabs or any unanswered questions. And making sure that all my assignments are posted. And then usually around that time, I started to see my research students, and so because they are undergraduates, they're taking a full course load, but they come in during specific hours and I talked to them about their plans for either the day or the week. I review their experiments and just make sure that they're on the right track. Or I may go to the lab and show them depending on the stage of undergrad. Whether they're new or they're more senior I may have to show them a particular technique. My day is also filled with meetings, especially as an administrator, I can have, I usually have at least one but sometimes I can have more. And then lots of impromptu meetings with either faculty and students. Just because of my position and I think physically where I see offices located. I see a lot of traffic in my office. So it's pretty demanding but it's fulfilling. I like to stay very busy. And so, my work life is very busy, and I enjoy that. I also have a family so I am married to another microbiologist and who is also a professor and who also has an administrative appointment. So our days are both very, very busy. But the benefit is that he works at the same institution and his office is right upstairs for mine. So we spend time together at work and we spend time together at home. And we also have two children, two daughters that are three and seven, and they are getting into different activities. So playing soccer, playing instruments, going to dance and so in addition to my hat that I wear as a at work, I wear a different hat in the evening and at night and just focusing on my family and also focusing on myself. I love to eat and my husband loves to cook so that balance works good but also making sure that I'm taking care of myself, either by working out, reading books is something that I have read implementing into my routine and just trying to find a balance between work and taking a break and enjoy my family. So it's not always a perfectly split, but it's times where I have to kick up the notch at work or pick up the notch at home. But overall, I think that finding a good balance between the two, even though it may not be on a day to day or week by week basis is really important. And it leads to a very just busy schedule sometimes.

Template created by Suann Yang and Rachel Pigg (2022)
Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

**Stephanie Mathews** 35:08
Thanks for sharing. That's exciting. Where did you grow up and what was it like where you grew up?

**Danielle Graham** 35:14
So I grew up between New York and North Carolina. I lived in New York for maybe about seven years and then I also lived in North Carolina and then I permanently moved to North Carolina in high school. And so New York, as you can imagine, was more fast paced. I did live in a more suburban area and not in what you typically think of in New York, but it's still, I think just New Yorkers in general have just a more fast paced life. Whereas North Carolina is just a little bit more relaxed. It has a more chill vibe, and being pretty much here since high school I did go on for graduate school for about five years. But I came right back to North Carolina because I love it so much. I will say that I am a North Carolinian and, surprisingly, both areas both New York and North Carolina in the areas that I've lived at, had very diverse populations. In New York, there was just a melting pot of different types of people. And in North Carolina, I live very close to Fayetteville when I was younger, and so Fayetteville is right next to what is now referred to as Fort Liberty prior was Fort Bragg. And you just see a lot of different types of people because of the military installation that is very close to us. So growing up in those two different environments were different, but similar and just being able to have both of those experiences I think was really important and just like shaping the way that I am now.

**Stephanie Mathews** 36:57
What keeps you motivated to do research?

**Danielle Graham** 36:59
The number one thing that keeps me motivated to do research is being able to work with undergraduate students. I think that when I was younger working in the lab was very attractive to me and I did enjoy it and I still do now but I definitely have moved past just wanting to do the research. It is more so morning to do the research in order to train the next generation. And so my role now I really, really enjoy because I'm able to contribute to students aspirations. I'm able to help them make their own discoveries and become passionate about research and just the potential of contributing to the society that really motivates me. I'm interested in the broader purpose of research and not maybe so much like the day to day experiments and learning different techniques and new techniques in order to make those contributions. I try to stay motivated, because again, undergraduates are very enthusiastic. And so just being able to set up different types of tasks and different types of experiments and celebrating all those small victories is really motivating for me to continue to do research.

**Stephanie Mathews** 38:22
Thanks so much for sharing. So thank you, Dr. Graham, for telling us about your research and about your background and steps to being a scientist and to where you are now.

**Danielle Graham** 38:31

Template created by Suann Yang and Rachel Pigg (2022)
Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms

Absolutely. I really enjoyed this. And it made me reflect on many, many things. And I hope that somebody learned at least a little bit about what it's like to be a scientist and also about antibiotic resistance and biofilm formation and the importance of those things.

Template created by Suann Yang and Rachel Pigg (2022)
Licensed under CC Attribution-NonCommercial-ShareAlike 4.0 International according to these terms