How do small things make a big difference?

Microbes, ecology, and the tree of life

Lesson 6: What can happen when my microbiome is disturbed?

I. Overview

Integrating concepts from previous lessons, this lesson extends students' thinking to consider what would happen to the composition of microbes in the human gut if a disturbance occurred. Building on ideas of microbial ecosystems, and the roles of microbes in the human body, students investigate a clinical trial in which patients with *C. difficile* infections (CDI) are treated with two different antibiotics. Students study data that show how the infection and antibiotics affect microbial communities in the human gut and how the disturbances connect to the recurrence rate of infection. Students then use their interpretations of the data as evidence to make and support conclusions about the best course of treatment for CDI. Finally, students further apply concepts of ecosystem dynamics and human-microbe interactions to compare two different approaches to treatment of bacterial infections: antibiotics and fecal transplants.

Connections to the driving question

The question of how microbes can affect the health of people is addressed in this lesson. Students explore how an imbalance in the microbiome can lead to illness and how a restoration of the balance can improve health.

Connections to previous lessons

This lesson draws on the ecosystem concepts and the technical knowledge covered in previous lessons. In the previous lesson, students are introduced to the experimental treatment of fecal transplants, created models of the human microbiome, and discussed roles of the various microbial communities in the microbiome. Now, they examine a "real life" situation where they must make a decision as to which antibiotics to use to treat *C. difficile* infection, a common occurrence in many hospitals. They must take into consideration the effects of these antibiotics on the microbiome, and the relation between gut microbiome recovery and human health.

II. Standards

National Science Education Standards

- Scientists conduct investigations for a wide variety of reasons. For example, they may wish to discover new aspects of the natural world, explain recently observed phenomena, or test the

conclusions of prior investigations or the predictions of current theories. (Grades 9-12 Understandings about scientific inquiry 2.2)
- Scientists rely on technology to enhance the gathering and manipulation of data. New techniques and tools provide new evidence to guide inquiry and new methods to gather data, thereby contributing to the advance of science. The accuracy and precision of the data, and therefore the quality of the exploration, depends on the technology used. (Grades 9-12 Understandings about scientific inquiry 2.3)
- Organisms both cooperate and compete in ecosystems. The interrelationships and interdependencies of these organisms may generate ecosystems that are stable for hundreds or thousands of years. (Grades 9-12 The Interdependence of Organisms 4.3)
- Human beings live within the world's ecosystems. Increasingly, humans modify ecosystems as a result of population growth, technology, and consumption. Human destruction of habitats through direct harvesting, pollution, atmospheric changes, and other factors is threatened current global stability, and if not addressed, ecosystems will be irreversibly affected. (Grades 9-12 The Interdependence of Organisms 4.5)

Benchmarks for Science Literacy

The Living Environment: Interdependence of Life
- If a disturbance such as flood, fire, or the addition or loss of species occurs, the affected ecosystem may return to a system similar to the original one, or it may take a new direction, leading to a very different type of ecosystem. Changes in climate can produce very large changes in ecosystems. (9-12 5D/H2)
- Human beings are part of the earth's ecosystems. Human activities can, deliberately or inadvertently, alter the equilibrium in ecosystems. (9-12 5D/H3)

III. Learning Objectives

Learning objective	Assessment Criteria	Location in Lesson
Create and defend models.	Student models should include enough information, visual and written, so that another individual can view the model and clearly identify the concept or process being represented. Students' final models will reflect accurate applications of ecology concepts.	Activity 1, 2 & 3

Learning objective	Assessment Criteria	Location in Lesson
Define and apply ecology concepts of diversity, disturbance, recovery, resilience.	**Diversity:** The measure of how many different species are in a given area. Diversity also refers to how much genetic difference there is in a given population. Typically, the greater diversity (in this case, number of species present in an ecosystem), the greater resiliency an ecosystem has. **Disturbance:** An event where the normal function and composition of an ecosystem are changed. The loss of a species or resource will disturb the ecosystem. The introduction of a species or resource will also disturb an ecosystem. **Recovery:** A process, or time period, where an ecosystem returns to its typical function and composition following a disturbance. **Resilience:** A measure of an ecosystem's ability to recover from a disturbance. An ecosystem is considered resilient if it is able to weather the disturbance and reorganize while retaining the same, or similar, function, structure, identity and feedbacks. An ecosystem with high resiliency would recover quickly. An ecosystem with very low resiliency may not be able to recover from a disturbance. Students will accurately apply these concepts as they discuss the activities and complete the student sheets.	Activity 1, 2 & 3

Learning objective	Assessment Criteria	Location in Lesson
Analyze and interpret graphs to evaluate the effects of two different antibiotics on the gut microbiome and in treating CDI.	Students are able to use the data and apply ecological concepts to compare and contrast vancomycin and fidaxomicin. Points of comparison include: • Both treatments reduce *C. difficile* counts to the same number. By day 40, *C. difficile* counts in vancomycin treated patients begin to increase and in fidaxomicin treated patients, the counts decrease. • For three of the four commensal microbe types measured, vancomycin kills more microbes than fidaxomicin. For two of these commensal microbe types, the counts in vancomycin-treated patients do not recover to the same level as those in the fidaxomicin-treated patients and are lower in count than the average count from healthy individuals. For these same two microbe types, counts for the fidaxomicin-treated patients are near those found in healthy individuals. • A fewer percentage of patients in the fidaxomicin group experienced recurrence than in the vancomycin group.	Activity 2
Practice making a claim and supporting it with evidence and reasoning to decide and defend an antibiotic treatment for a *C. difficile* infection.	Students clearly articulate a claim that states which antibiotic is a better treatment for CDI. They provide appropriate and sufficient evidence (data) to support their claim. They provide appropriate and sufficient reasoning that uses scientific principles to link the evidence to the claim. Students apply concepts of diversity, disturbance, recovery, resilience, and competition in their reasoning.	Activity 2

Learning objective	Assessment Criteria	Location in Lesson
Compare and contrast different treatment methods for a *C. difficile* infection.	Fecal transplants and antibiotics take two different approaches to treating *C. difficile* infections and are at different stages of development. Fecal transplants add commensal microbes to the gut microbiome to reinstate the microbiota that was lost in the original disturbance. This method treats the infection by making it difficult for the *C. difficile* to compete with the commensal microbes for space and resources. Fecal transplants are a newer and, thus far, less extensively tested treatment. Patients may be hesitant to use this treatment because of the ick-factor associated with fecal matter. With antibiotic treatment, the goal is to eliminate or significantly reduce the *C. difficile* population to treat the infection. By reducing the number of *C. difficile,* the healthy gut microbiome has an opportunity to recover. However, antibiotics can kill other commensal microbes along with the *C. difficile.* Antibiotic treatment has been widely used for much longer than fecal transplants. Patients may be more familiar and, therefore, more comfortable with antibiotic treatment.	Activity 3

IV. Adaptations/Accommodations

This lesson requires students to analyze and interpret graphical data, which may be difficult for students who are not familiar with reading graphs. As a class, or in small groups, a discussion can be held to identify what the different X- and Y-axes represent and what each line represents. Also, the interpretation questions can be modified to provide more scaffolding for students. The final recommendation of antibiotic treatment where students develop an argument based on evidence can also be created as a whole class, as opposed to having students work as individuals or in small groups.

The section on PCR (a paragraph in Case Review Part B and U9_L6_Resource_PCR.docx) has been included as enrichment for advanced students or classes. It may be skipped without detriment to the conceptual flow of the lesson.

Safety

There are no additional safety concerns associated with this lesson.

V. Timeframe for lesson

Opening of Lesson

- Discussion linking to concepts learned in previous lesson– 10 minutes

Main Part of Lesson

- Activity 1: What is a microbiome disturbance? – 20 minutes
- Activity 2: Case study of recurrent *C. difficile* infections – 80 minutes
- Activity 3: Comparison of treatment methods for *C. difficile* infections – 30 minutes

Conclusion of Lesson

- Closing discussion on unit driving question – 10 minutes

VI. Advance prep and materials

Activity 1: What is a microbiome disturbance?

Materials:

- Images of the interior of the gut and graphs of three healthy subjects treated with antibiotics (*U9_L6_Images_AntibioticRecovery.pptx*)
- What is a microbiome disturbance? student sheet *(U9_L6_StudentSheet_WhatIsMicrobiomeDisturbance.docx) : 1/student*
- Save the image "Intestinal Bacteria" found at the following link: http://ngm.nationalgeographic.com/2013/01/125-microbes/oeggerli-photography#/05-intestinal-bacteria-670.jpg

Preparation:

- Queue PowerPoint images to be used in resiliency and recovery discussion.
- Photocopy "What is a microbiome disturbance?" student sheet – 1/ student.
- Add the image "Intestinal Bacteria" to the first slide of the PowerPoint presentation: *U9_L6_Images_AntibioticRecovery.pptx*

Activity 2: Case study of recurrent *C. difficile* infection

Materials:

- Case Review Part A (*U9_L6_CaseReview_PartA.docx*): 1/student or group (class set)
- Case Review Part B (*U9_L6_CaseReview_PartB.docx):* 1/student or group (class set)
- Student Sheet Part A (*U9_L6_StudentSheet_PartA.docx):* 1/student
- Student Sheet Part B (*U9_L6_StudentSheet_PartB.docx):* 1/student
- Student sheet Part C (*U9_L6_Student Sheet_PartC.docx):* 1/student
- Base CER(R) Rubric for evaluating students' CERs, (U9_L6_*Resource_BaseRubric_CERR.docx*)
- PCR and DNA Sequencing resource, **optional** (*U9_L6_Resource_PCR.docx*)

- Poster paper or whiteboards
- Markers

Preparation:

- Copy Case Reviews and put them into individual folders labeled "Case Review: Part A" and "Case Review: Part B" to give to each group.
    - Note: Once students complete Student Sheet Part A, they will return Case Review A so they can receive Case Review B.
- Copy Students Sheets Part A, Part B, and Part C
- (Optional) Copy Base Rubric for CER and PCR and Dana Sequencing resource

Activity 3: Comparison of treatment methods for *C. difficile* infections

Materials:

- Student Sheet: Hospital Memorandum *(U9_L6_StudentSheet_HospitalMemorandum.docx):* 1/group
- Poster paper or whiteboards
- Markers

Preparation:

- Copy Hospital Memorandum student sheet
- Gather markers, poster board, etc.

VII. Resources and references

Teacher resources

- For a review paper on the resiliency and recovery of the human microbiome: Relman, D. (2012). The human microbiome: Ecosystem resilience and health. *Nutrition Reviews*. (Suppl. 1): S2-S9.
- For more information on the claim, evidence, and reasoning framework for scientific explanations: McNeill, K. L. & Krajcik, J. (2012). Supporting grade 5-8 students in constructing explanations in science: The claim, evidence, and reasoning framework for talk and writing. New York, NY: Pearson Allyn & Bacon.
- The graphs in Part 2 of the student sheet "What is a microbiome disturbance?" (Activity 1) are from: Dethlefsen, L. and Relman DA. (2011). Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc Natl Acad Sci*. 108(Suppl. 1): 4554-4561.
- The case study in Activity 2 draws its data from the clinical trial described in the paper: Louie, T.J., Cannon, K., Byrne, B., Emery, J., Ward, L. Eyben, M. & Krulicki, W. (2012). Fidaxomicin preserves the intestinal microbiome during and after treatment of *clostridium difficile* infection

(CDI) and reduces both toxin reexpression and recurrence of CDI. *Clinical Infectious Diseases*, 55 (S2), S132-S142.
- The reading in Activity 3 is a modified excerpt from: Rowan, K. (2012). “Poop Transplants” may combat bacterial infections. Live Science. Retrieved from http://www.livescience.com/36701-poop-transplants-bacterial-cdiff-infections.html

References

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VIII. Lesson Implementation

Opening of Lesson:

Begin the lesson by asking students to review what they learned about the human microbiome in the previous lesson.

- How are microbes beneficial to our bodily functions?
    - Students should be able to provide examples from the readings in the previous lessons.
- What can happen if the microbiome is disrupted? If native microbes are killed? Or if a harmful microbe starts to grow where it doesn't belong?
    - Possible student responses include that an infection can occur, or potentially allergies and/or autoimmune diseases can occur, if certain native microbes are not present in/on the body.

Ask the students who read the article on fecal transplants to describe what a *C. difficile* infection (CDI) is and how it can occur. Guide students to cover the following in their explanations of the article and also review these points with the whole class:

- A *C. difficile* infection often occurs when the gut microbiome is already disturbed and has fewer quantity and types of native microbes. This type of disturbance leaves the gut microbiome vulnerable to "invasion" by *C. difficile* which takes advantage of the now available space and resources. Patients with CDI are usually treated with antibiotics but a new treatment, fecal transplant, is being piloted to rid patients of the infection by repopulating the gut with native microbes. Native microbes re-introduced by the transplant out-compete the *C. difficile* for space and nutrients, thus decreasing the number of *C. difficile*.

Once students have a clear understanding of what a *C. difficile* infection is, tell them that they will review a case study of patients infected with *C. difficile* where, based on study results, they have to decide which antibiotic will be used. Since fecal transplant is a relatively new method of treatment, doctors still rely primarily on antibiotics to clear microbial infections. The antibiotic treatment itself, however, can cause further disturbance in the already disrupted gut microbiome. Thus, students will have to analyze and interpret data to determine which treatment will best clear the *C. difficile* infection.

Crosscutting Concepts: Stability and Change

Stability and change is a key crosscutting concept throughout this lesson. The microbial ecosystem of the human gut, like most ecosystems, is in dynamic equilibrium, where various disturbances (both major and minor) occur over time. This natural fluctuation can be seen in the graphs (microbial diversity fluctuates prior to antibiotic administration) in Part 2 of the "What is a microbiome disturbance?" student sheet in Activity 1. There are also instances of more significant changes such as when an ecosystem is disturbed. Following an ecological disturbance, an ecosystem is

	considered resilient if it is able to recover to its original structure and function. In this lesson, students will complete a case study that allows them to explore the concepts of stability and change through an example of the gut microbiome's response to a disturbance and what it can mean for human health.

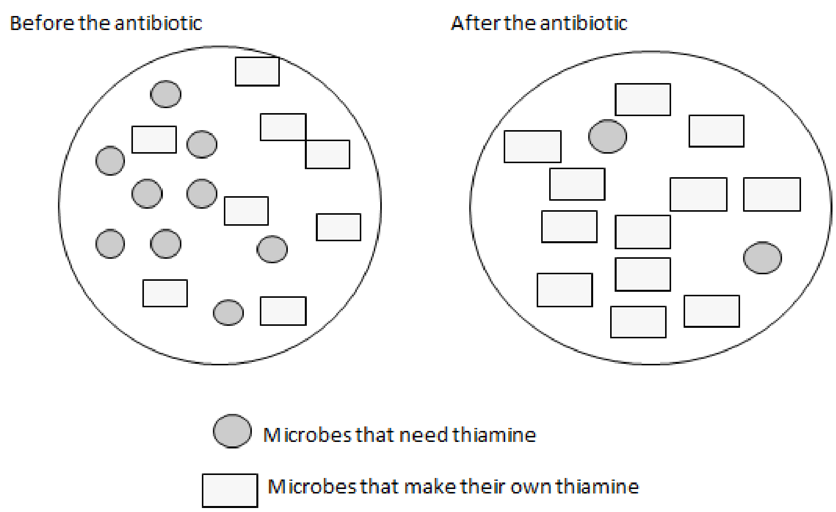
Main Part of Lesson

Activity 1: What is a microbiome disturbance?

Explain to students that before getting to the case study, they must first learn more about ecological disturbances and how to interpret graphs showing effects of antibiotics on gut microbes. To begin the activity, pass out the student sheet (*U9_L6_StudentSheet_WhatIsMicrobiomeDisturbance.docx).* Show students the first image on the PowerPoint (*U9_L6_Images_Antibiotic Recovery.pptx*). Allow students some time to work on Part 1 by themselves or in small groups before discussing the questions as a whole class. This should be a refresher of the concepts from previous lessons and an extension into the specific environment of the gut. The first image is designed to visually demonstrate that there are many different types of microbes in the gut. A key point is that some microbes are beneficial, some harmful and some neutral in their effects on humans; most of the native microbes do not harm humans.

Teacher Pedagogical Content Knowledge

Students are asked a number of times throughout the lesson to draw a model. It is expected that they will create an image similar to the one below. This one specifically addresses Question 6 of the student sheet "What is a microbiome disturbance?". In some cases, such as this one, students will need to draw and compare two images or to draw a series of images to explain what is happening. Encourage students to label and explain their models thoroughly so that another person is able to understand it.



After reviewing Part 1 of the student sheet, show slide 2 of the PowerPoint which has graphs of the effect of antibiotics on the microbiomes of three subjects. The graphs come from a published article and illustrate the microbial diversity found in a healthy human gut, the effects of antibiotic use on microbe diversity, and the microbial community's resilience (Dethlefsen & Relman, 2011).

Begin Part 2 of the student sheet by first orienting students to key features of the graphs. Draw students' attention to the different graph components highlighted in the figure caption on the student sheet.

Teacher Content Knowledge

The graphs in Part 2 of the student sheet (*U9_L6_StudentSheet_WhatIsMicrobiomeDisturance.docx)* come from an article published in the journal *PNAS* by Dethlefsen & Relman (2011). The following is a brief explanation of the graphs based on descriptions provided in the article.

In this study, scientists were interested in looking at how human gut microbial communities are affected by disturbances. To investigate this, they gathered data on the normal distal gut microbiota of three healthy adults and its response to two instances of antibiotic administration. The researchers collected fecal samples for two months before administering the broad-spectrum antibiotic, ciprofloxacin, on day 60 (as indicated by the first bold line on the graphs). Researchers continued to collect fecal samples for six months after the first antibiotic course. Six months after the initial antibiotic treatment, researchers administered ciprofloxacin again (as indicated by the second bold line on the graphs) and collected fecal samples for two more months after the second antibiotic treatment.

The graphs in Part 2 of the student sheet show the data that was collected. Study participants' fecal samples were analyzed for "taxon richness" (indicated on the left y-axis of the graphs) which is the total number of microbial refOTUs (OTUs= operational taxonomic units that are groups of related organisms). The fecal samples were also analyzed for "phylogenetic diversity" (indicated on the right y-axis) which is the number of members in each refOTUs as well as the total number of refOTUs. Low phylogenetic diversity could indicate that there are many refOTUs present but some of refOTUs do not have many members; it could also indicate that there is a low number of refOTUs present. In contrast, high phylogenetic diversity indicates that there are many refOTUs as well as many members in each refOTU. The x-axis shows the experiment day on which the sample was collected.

Depending on the level of students in the classroom, when explaining the y-axis of the graphs, the taxon richness and phylogenetic diversity can be more simply explained as

	two different ways to measure the diversity of microbes. Reference: Dethlefsen, L. & Relman, D.A. (2011). Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc Natl Acad Sci USA*. 108(Suppl. 1): 4554-4561.

After students have worked together to answer questions in Part 2, lead a class discussion to review the main concepts. Use the questions in the student sheet and the following questions to guide the discussion. Throughout discussion, reinforce the following terms: diversity, disturbance, recovery, and resilience. Some students may not know these terms yet, but by analyzing the graphs and answering the questions, they have already begun to work with these important ecological concepts.

- What information are these graphs communicating?
    - How an antibiotic treatment affected the microbiome of three different healthy people.
- What do you notice about the microbial diversity of the fecal samples before the first antibiotic treatment? How do the three subjects compare with each other?
    - There are fluctuations in these values before antibiotic treatment, indicating that the gut microbiome is constantly in some measure of flux. Each subject also begins with different values, indicating that there are individual differences in the diversity and number of microbes in each person's gut.
- What happened to the microbial diversity of each subject's gut microbiome after the first antibiotic treatment?
    - First, the values decrease greatly right after antibiotic treatment, indicating a decrease in **diversity** and numbers of microbes in the gut. The antibiotic treatment caused a **disturbance** to the existing microbial community.
    - Then, the microbes begin to **recover**—increasing in diversity toward the level that existed before the antibiotic treatment. This indicates the microbial community is **resilient** and can re-establish the original structure and function of the ecosystem.
- What happened to the microbial diversity of each subject's gut microbiome after the second antibiotic treatment?
    - The antibiotic treatment disturbed the microbial community again and caused a decrease in the number and diversity of microbes in the gut microbiome.
    - The microbial diversity did not recover as quickly as after the first antibiotic treatment, particularly in subject A (Graph A).

During the discussion, make sure to review with all students the effects of an ecological disturbance on diversity and the role of diversity in an ecosystem's resilience and recovery. These concepts will be important as students work through the case study in Activity 2.

Lastly, for Part 3 of this activity, have students work together in groups to define the terms: diversity, disturbance, recovery, and resilience. This can also be done together as a class. To do this, students should draw on Parts 1 and 2 of the student sheet and the discussions they have been having thus far in the lesson. Along with the definitions, students should also indicate examples of each of the terms. Review the definitions as a class to make sure all students have an accurate and common understanding of the terms. The definitions should look like this:

- **Diversity:** The measure of how many different species are in a given area. Diversity also refers to how much genetic difference there is in a given population. Typically, the greater diversity (in this case, number of species present in an ecosystem), the greater resiliency an ecosystem has.
- **Disturbance:** An event where the normal function and composition of an ecosystem are changed. The loss of a species or resource will disturb the ecosystem. The introduction of a species or resource will also disturb an ecosystem.
- **Recovery:** A process, or time period, where an ecosystem returns to its typical function and composition following a disturbance.
- **Resilience:** A measure of an ecosystem's ability to recover from a disturbance. An ecosystem is considered resilient if it is able to weather the disturbance and reorganize while retaining the same, or similar, function, structure, identity and feedbacks. An ecosystem with high resiliency would recover quickly. An ecosystem with very low resiliency may not be able to recover from a disturbance.

Activity 2: Case study of recurrent *C. difficile* infection

Once students have an understanding of ecological disturbances within the context of the human gut, they now play the role of medical investigators charged with evaluating different treatments for a *C. difficile* infection. They will be presented with information gathered by Dr. Rivera (a fictional character), a medical researcher who has developed a new drug, fidaxomicin, in order to treat recurrent *C. difficile* infections. Recurrent CDI is a growing problem at Central Midwest Hospital (a fictional hospital) and Dr. Rivera has been working on an antibiotic that can kill the *C. difficile* while disturbing the gut microbiome less than other antibiotics.

Teacher Pedagogical Content Knowledge

The data students are given to work with in the case study comes from a study done by Louie et al., 2012. The representations of data have been recreated and modified to be more accessible for high school students. However, the data in the student sheets is authentic in that it accurately reflects data presented in the published paper. Dr. Rivera and Central Midwest Hospital, however, are a fictional doctor and hospital used to tell the story of this case study.

Reference: Louie, T.J., Cannon, K., Byrne, B., Emery, J., Ward, L. Eyben, M. & Krulicki, W. (2012). Fidaxomicin preserves the intestinal microbiome during and after treatment of *clostridium difficile* infection (CDI) and reduces both toxin reexpression and

	recurrence of CDI. *Clinical Infectious Diseases*, 55 (S2), S132-S142.

Ask students if they have ever heard of the term "clinical trial" and know what it is. After taking some student responses, explain the following to the students:

- A clinical trial is a type of experiment that is often used to test new medicines or other health-related interventions. The purpose is to figure out the effects of the new medical product to determine whether it is good, safe, and effective at treating a condition. Investigators of a clinical trial determine the research protocol of the trial and participants are assigned to either a control or treatment group. The control group receives a placebo, no treatment, or an already proven drug/ procedure. The other group(s) receives the drug/treatment/device of interest. Results from the two groups are compared to see if there is a significant difference between their outcomes. This type of testing is required by law before any new drug, treatment or device may be used by the general public (Meinert, 1986).

To begin the case study, arrange students into groups of three or four. Hand each group a folder with the Case Review Part A in it (1 copy per 1-2 students). Each student should also receive Student Sheet Part A. After reading the Case Review as a group, students should work together to answer the follow-up questions in Student Sheet Part A. Case Review Part A explains the background and set up of the clinical trial that is the focus of this case study. The questions that follow ask students to draw models of a typical gut microbiota and a *C. difficile* infected gut microbiota. Students are then asked to use models and words to articulate Dr. Rivera's hypothesis for the outcome of the trial.

	Scientific Practices: Analyzing and Interpreting Data In Part B of the case study, students work with real data from the scientific paper published by Louie at al., 2012. Students will need to apply their knowledge of ecological concepts to pick out patterns from line graphs and interpret data in pie charts. Student Sheet Part B will guide students through this data analysis and interpretation, drawing students' attention to particularly salient features of the data. At the end of the case study students will need to use this data as evidence to make and support their conclusions.

Once students have completed Part A, they return Case Review Part A to receive Case Review Part B and Student Sheet Part B. Case Review Part B explains the procedures used for data collection and the type of data collected. It also includes several graphs that document the results of the trial. After reading the Case Review, students work in their groups to answer questions in Student Sheet Part B. These questions will guide the students through the data presented in the Case Review and help them to analyze and interpret the graphs. As students work, walk around to help answer questions and facilitate discussion in the student groups. Encourage students to use the terms diversity, disturbance, recovery, resilience, and competition in their discussions and written answers.

Cross Cutting Concepts: Scale, Proportion and Quantity

It may be necessary to discuss with students what the X- and Y-axes represent on the graphs. On the y-axis, the number of CFUs (colony forming units) is in log10 scale. That means that when it says "2" on the Y-axis it really means 10^2, or 100. This represents the number of bacteria present in the sample. The X-axis represents at which day the researchers collected samples. One line of the graph represents the group treated with vancomycin and the other line the fidaxomicin-treated group. The values for each group are an average of the 10 patients treated with either fidaxomicin or vancomycin whose fecal samples were analyzed for microbial composition.

After all students have completed Student Sheet Part B, except for the final question, have a class discussion to see how each group interpreted the graphs. Some groups may have arrived at different conclusions based on the data. Ask students questions from the Student Sheet such as the following:

- Based on the *C. difficile* counts, would you expect there to be more recurrent infections in the vancomycin or fidaxomicin? Explain your answer.
    - It is hard to tell. The levels of *C. difficile* are about the same for both antibiotic groups. However, at the end of the time indicated in the graphs, *C. difficile* counts for the vancomycin group start to increase and start to decrease for the fidaxomicin group.
- Based on the commensal microbes' graphs, would you expect there to be more recurrent infections in the vancomycin or fidaxomicin? Why?
    - Would expect to see more recurrent infections in the vancomycin group because more of the mutualistic microbes were killed and therefore, there was less recovery of the populations. Since the native microbes have not recovered, *C. difficile* will have more opportunities to recolonize the gut. The final counts of commensal microbes in the fidaxomicin group are closer to those of the healthy averages.

After students have shared their ideas, and students have a good grasp on what the data is showing, give students time to complete the final part of Student Sheet Part B. This last question asks students to revisit their initial models that they developed in Part A and to revise them based on the new information they gathered through the case study. Students should work in their groups to discuss the data and findings and how this impacts their revision of the models of how vancomycin and fidaxomicin affect the native gut microbes and *C. difficile*.

Teacher Pedagogical Content Knowledge

The data presented in this lesson highlight the importance of considering multiple possible direct and indirect effects of antibiotics. It is important to help students to recognize that this case study illustrates a small portion of the much larger and highly complex science of the effects and consequences of antibiotic use. Students should be encouraged to continue to consider the potential advantages and disadvantages of antibiotic use and to acknowledge the complexity of the issue.

Once students have developed their revised models, hand out Student Investigation Sheet: Part C. This last part of the case study asks students to make a decision and provide an explanation as to which antibiotic is best suited for treatment of *C. difficile* infections. Students are asked to make a claim (which antibiotic is better suited for treatment), support their claim with evidence (data) and provide reasoning (the scientific principles that connect evidence and claim). This can be an individual, group, or whole class activity depending on the student level.

Have students work in small groups to create a poster (or draw/write on whiteboards) to display their scientific explanation/argument. Then, have each group share their posters. Encourage students to ask questions and critique posters of other groups. This is a good opportunity to push student thinking and make sure that their reasoning clearly links the evidence to the claim.

Teacher Pedagogical Content Knowledge

Scientific explanations/arguments need to include a claim (C), evidence (E) that support that claim, and reasoning (R) that link the claim and evidence. Reasoning is based on known scientific principles that make the evidence relevant to the claim.

If this is the first time students are using the CER framework, it may be necessary to complete a CER as a whole class. This can be done with writing the terms “claim,” “evidence,” and “reasoning” at the front of the room, instructing students what these terms mean, and then having students share what they think is a claim, evidence, and reasoning for determining which antibiotic to use. A format for supporting students in this process is shown below. This format encourages students to clearly link each piece of evidence (there should be multiple) with reasoning.

Claim:	
Evidence: 1. First piece of evidence that supports claim.	Reasoning: 1. Explanation of why evidence 1 actually supports the claim.

Once students are able to use the CER framework effectively, students can be

	encouraged to go a step further to include a rebuttal in their explanation, making it a CERR. A rebuttal would be a statement describing why another claim is not as "good" as the claim being championed in the explanation by the student. Each rebuttal could include evidence and reasoning as well. A Base CERR Rubric is also available to hand out to students to inform them on how their CERR will be evaluated. It is recommended that this rubric be modified for the activity and the particular set of students. For more information on how to support students in writing CERs, refer to: McNeill, K. L. & Krajcik, J. (2012). Supporting grade 5-8 students in constructing explanations in science: The claim, evidence, and reasoning framework for talk and writing. New York, NY: Pearson Allyn & Bacon.

Activity 3: Comparison of treatment methods for *C. difficile* infections
In this activity students will compare two different *C. difficile* treatment methods in order to apply their knowledge of ecosystem dynamics. One method is antibiotic use (covered thus far in the lesson) and the other is fecal transplants, which were introduced in the previous lesson and reviewed at the beginning of this lesson.

Arrange students in small groups (these can be the same groups students were already working in or new groups). Hand out the Hospital Memorandum student sheet (*U9_L6_StudentSheet_HospitalMemorandum.docx).* The first page is an excerpt from the fecal transplant reading in the previous lesson. Though some students will have already seen this information, it will be helpful for them to quickly review. The second page has a set of questions to guide students in creating a visual organizer to compare and contrast the two treatment methods. While students can use a venn diagram to compare treatments, there are many other ways to organize the information. Students should be encouraged to use any method that works for them and accomplishes the task. Emphasize to students that a model demonstrating how each treatment works would be very useful in this comparison and that all organizers should include them.

When students have completed their treatment comparison, have some students or groups of students present their treatment method comparisons to the class. Use the presentations and the questions from the student sheet to lead a class discussion about the ecological concepts that underlie antibiotic and fecal transplant treatments.

	Crosscutting Concept: Cause and Effect Throughout the lesson, students relate causes and effects. This models the behavior of scientists in that one of scientists' primary goals is to study causal relationships and the mechanisms by which they operate. In the case study, students relate different treatments (different antibiotics as well as different treatment methods) to their likely

	outcomes based on their understanding of ecological principles that underlie this connection. Being able to provide the scientific reasoning that explains the link between a cause and its effect is a particularly important aspect of understanding this crosscutting concept in science. While students should investigate cause and effect relationships, encourage students to also be cautious in how they describe the phenomena and the conclusions they come to. Data can often show correlational relationships and not conclusive causal relationships. Rigorous and multiples tests are critical for determination of causes and effects.

Conclusion of Lesson

Conclude the lesson and the unit by leading a big picture discussion about what students learned throughout the unit. Use questions such as the following to facilitate the discussion:

- Have your ideas on our interactions with microbes changed?
- What facts/ideas have surprised you or challenged your own ideas about microbes in this unit?
- Can our body be considered an ecosystem? Why or why not?
    - Throughout their answers and explanations, encourage students to use the ecological terms they have encountered throughout the unit (organism, population, community, habitat, niche, competition, disturbance, recovery, resilience).
- How do you think Carl Woese's three domain tree of life model has affected our understanding of microbes?

Homework and Assessment

The "What is a microbiome disturbance?" student sheet may be done as homework in preparation for this lesson. Student Sheets Part A and Part B should be completed in class and Student Sheet Part C can be completed in class or as homework. Student Sheet Part A assesses students' abilities to develop and use a model to create a hypothesis. Student Sheet Part B assesses students' abilities to analyze and interpret data as well as revise their model based on new evidence. Student Sheet Part C assesses students' abilities to create a scientific explanation/argument using the claim, evidence, reasoning (CER) framework. These items and the student product for Activity 3 assess students' ability to again apply general knowledge of ecosystem principles and concepts to specific examples.

An optional final assessment for the end of the unit can be a scientific explanation using the CER framework that answers the driving question of the unit "How do small microbes make a big difference?" Students can use multiple pieces of evidence from throughout the unit regarding microbial roles in various ecosystems. They can also use evidence from the first lessons describing how Carl Woese's discovery, using DNA sequencing, led to a significant increase in research about how small microbes make a big difference.