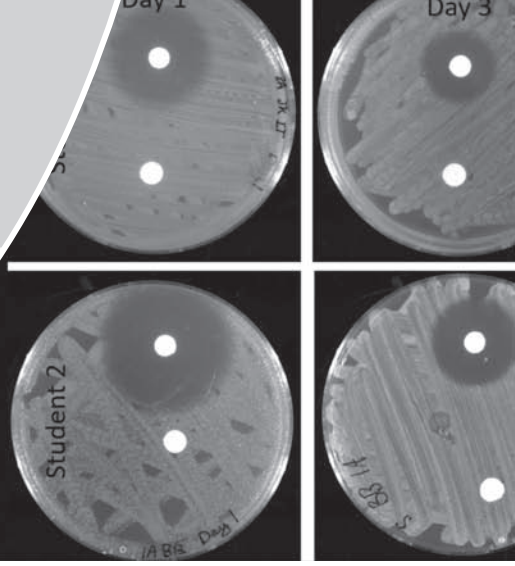
INQUIRY & INVESTIGATION

Modeling the Emergence of Antibiotic Resistance in Bacterial Populations

RECOMMENDED FOR *AP Biology*



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Abstract

Since antibiotics have become routinely used to treat infections, antibiotic resistance is now an emerging concern for public health. To understand how bacteria become resistant to antibiotics, many students draw from the common misconception that bacteria gain resistance upon antibiotic exposure. We have designed models and a corresponding lab that explores how a population of bacteria can evolve antibiotic resistance, with emphasis on dispelling common misconceptions surrounding the mechanism of antibiotic resistance. Using an antibiotic disk diffusion assay, students compare the antibiotic resistance level of a harmless E. coli *strain of bacteria over time. Then, students compare their lab data to the models, which together illustrate the roles that initial genetic variation and random mutation play in the evolution of antibiotic resistance. In this guided investigation, basic microbiology concepts and techniques are made accessible to students in a high school classroom. The models developed here are in line with the practices of the* Next Generation Science Standards (NGSS). *The models, together with the lab, are used to guide students through the process of argumentation using a claim, evidence, and reasoning (CER) format to explain the evolutionary mechanisms of antibiotic resistance.*

Key Words: *microbiology; evolution; modelling; antibiotic resistance; bacteria.*

○ Introduction

It is widely accepted that evolution is central to understanding all other aspects of biology (Dobzhansky, 1973); however, teaching evolution can be conceptually challenging (Smith, 2010). Thus, it is not surprising that a recent survey revealed that almost half of teachers are spending fewer than three class periods teaching key topics in evolutionary biology. Teachers cited a lack of good laboratory exercises and supplemental materials as a major obstacle to effective teaching of evolution (Romine et al., 2014; Griffith & Brem, 2004; Friedrichsen et al., 2016). Therefore, we have developed an investigation that illustrates the phenomenon of natural selection using the example of emergence of antibiotic resistance in a bacterial population. This investigation incorporates the *Next Generation Science Standards* (*NGSS*) high-leverage practices of scientific modeling and argumentation. Antibiotic resistance is often taught as a black box—students are taught that bacteria survive or die in the presence of an antibiotic; however, there is no discussion of the underlying mechanism(s) of antibiotic resistance. In this investigation, we highlight several mechanisms of antibiotic resistance that are easy for students to understand based on their prior knowledge of cell structure, function, and processes. We use this investigation to introduce a larger unit on natural selection taught in a tenth-grade honors biology course, and the students have already been introduced to the peppered moth example in middle school.

Bacteria are an attractive model organism for teaching evolution because natural selection can be observed on a short time scale.

Bacteria are an attractive model organism for teaching evolution because natural selection can be observed on a short time scale, due to the rapid growth rate of bacteria. In addition, antibiotic resistance provides a contemporary example of evolution that will promote student engagement. Despite being a hot topic in the media, a clear understanding of antibiotic resistance is severely lacking in the general population. A study conducted by the World Health Organization found that adults realize antibiotic resistance is a problem, but don't fully understand what causes it (WHO, 2015). Although disc diffusion assays are widely used in lab exercises to classify antibiotic resistance, the bacteria are typically classified as sensitive or resistant. In this module, students observe antibiotic resistance as a trait that changes over time due to accumulation of random mutations. This analysis is coupled with introducing students to the mechanisms bacteria use to become antibiotic resistant. Completion of this lab module enables wide-ranging discussions of the socio-scientific issues surrounding antibiotic resistance including the

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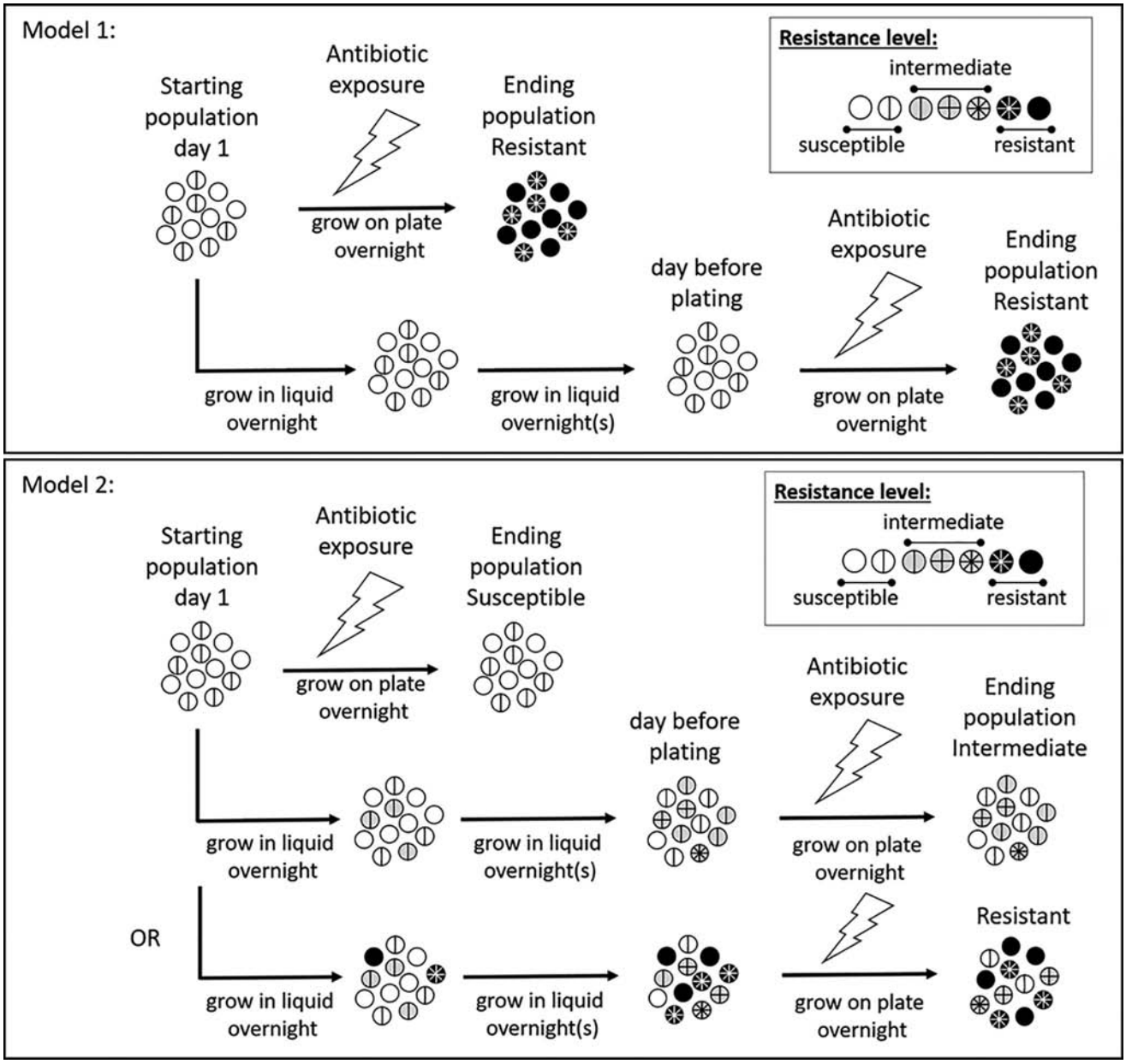

Figure 1. Models of antibiotic resistance emerging in a population. In both models, the white circles depict a susceptible population of bacteria that is either grown overnight on a plate with antibiotics (top) or grown for several days in liquid and then grown overnight on a plate with antibiotics (bottom). Model 1 represents the needs-based acquisition of antibiotic resistance. Here, bacteria become antibiotic resistant (black circles) when they are exposed to the antibiotics. Model 2 illustrates the acquisition of antibiotic resistance through random mutation independent of antibiotic exposure. On day 1 the population will remain susceptible (white circles, top row) because mutations have not accumulated. If mutations leading to antibiotic resistance are acquired later during growth, the population may have an intermediate level of resistance (middle row). If antibiotic resistance is acquired early during growth (black circles, bottom row), the population will become resistant.

overuse of antibiotics, agricultural practices, and the emergence of herbicide-resistant plants.

One challenge to teaching microbiology in a classroom setting is that microorganisms are invisible to the naked eye, which is a barrier to understanding bacteria-related concepts. For this reason, we have developed a set of two models that make bacterial populations visible to students. Students can use these models to help conceptualize microbiology and evolutionary concepts, and reason with the models to promote their understanding of the antibiotic resistance laboratory investigation. In using the scientific models to reason, students are engaged in the authentic practices of scientists. Evaluation of the models helps students rule out the needs-based model of evolution (Figure 1, Model 1), which is a common misconception and barrier to understanding evolution (Bishop & Anderson, 1990; Alters & Nelson, 2002). As a final activity, students write an argument explaining which model is best supported by their data.

Here we present a laboratory module that provides teachers with additional tools to teach evolution, allows students to directly experience evolution in the context of microbiology, and actively engages students by drawing on current topics such as the emergence of superbugs.

Student Objectives

1. Identify prokaryotic cell structures and their functions.
2. Define antibiotics and selective toxicity.
3. Describe how antibiotics work to kill bacteria.
4. Explain mechanisms bacteria use to evade antibiotics.
5. Identify aspects of natural selection, specifically random mutation resulting in genetic variation, selective pressure, and differential survival and reproduction.
6. Evaluate the merits of two models to explain the results of the investigation.
7. Explain how and why bacteria become resistant to antibiotics.
8. Demonstrate the following microbiology laboratory skills: sterile technique, culturing bacteria, and antibiotic disk diffusion assay.

Alignment with *Next Generation Science Standards* (*NGSS*)

This lab module highlights two high-leverage *NGSS* science practices, scientific modeling and argumentation, and includes the practices of carrying out an investigation, analyzing, and interpreting data. The modeling activity aligns with the *NGSS* modeling learning progression for grades 9–12, which states that students should be able to "evaluate merits and limitations of two different models of the same proposed tool, process, mechanism or system to select or revise a model that best fits the evidence or design criteria" (NGSS Lead States, 2013, App. F, p. 6). In the past, concrete scale models such as DNA models and fluid mosaic membrane models (Harrison & Treagust, 2000) have been used to make molecular and cellular structures visible to students. In these examples, students do not view a model as a tool to generate new knowledge, but as a *means of showing others what the phenomenon looks like* (Schwarz et al., 2009, p. 640). Thus, we wanted to expand our use of models from concrete scale models to "generative tools for explaining and prediction" (Schwarz et al., 2009, p. 639). The models developed here (Figure 1) are used as tools to help students think and reason about evolutionary phenomena in the context of microbiology. At the end of the module, students write an argument, using a claim, evidence, and reasoning (CER) format (Toulmin, 1958), defending their choice of Model 1 or Model 2 as best explaining why bacteria acquire antibiotic resistance. The argumentation activity aligns with the *NGSS* argumentation learning progression for grades 9–12, which states students should be able to "construct, use, and/or present an oral and written argument or counter-argument based on data and evidence" (NGSS Lead States, 2013, App. F, p. 13).

○ Introducing the Investigation

Prior to the laboratory investigation, we show a 9-minute video clip from the Frontline video, "Hunting the Nightmare Bacteria," which introduces the issue of methicillin-resistant *Staphylococcus aureus* (MRSA) through the story of an adolescent girl who has a MRSA infection (Young, 2013). We elicit students' prior knowledge and experiences with MRSA and explain that we will be investigating antibiotic resistance using *Escherichia coli*, a harmless rod-shaped bacterium that colonizes the guts of living organisms, in place of the dangerous MRSA (PowerPoint slides 1–3).

At the start of the module, students are introduced to several key concepts, beginning with a background on antibiotics, bacterial cell structures, and the mode of action of nalidixic acid (Student Handout 1, PowerPoint slides 4–14). Students are then given instructions for the lab exercise (Student Handout 2), and the lab exercise is started. Prior to completing the lab exercise, collecting data, and analyzing results, students are introduced to mechanisms used by bacteria to gain antibiotic resistance (Student Handout 3, PowerPoint slides 15–29).

○ Details of the Lab Module

The module is carried out over five class periods; days 1, 3, and 5 are student work days, and days 2 and 4 require minimal work from only the teacher (Table 1, Figure 2). Students work in small groups of 2 or 3 to complete the tasks involved in this lab module. During day 1, students resuspend a colony of bacteria in growth media. They use this cell suspension to swab an agar plate with a lawn of bacteria and start a liquid culture of bacteria. Finally, the students place two disks on the plate: a blank disk and an antibiotic disk. An overview of this procedure is illustrated in Figure 2A, and the step-by-step procedure is provided in Student Handout 2, page 1.

The plates are incubated at 37°C for one day, then moved to the refrigerator (4°C). The day 1 student lab portion can be completed in approximately 45 minutes. Next, students are introduced to how the antibiotic disk diffusion assay works and to key terms in antibiotic resistance such as "susceptible," "intermediate," and "resistant" (PowerPoint slides 16–22). Students make predictions for where bacterial growth will occur if a bacterium is resistant or susceptible to the antibiotic, to illustrate their understanding of the disk diffusion assay and the expected results (Student Handout 2, p. 3).

On day 2 of the lab, the teacher subcultures the student's test tube of bacteria by transferring 100 µL of *E. coli* from the student's tube into a new test tube with fresh media, and places the tube in the shaking incubator (Figure 2B). Student plates are removed from the incubator and placed in the refrigerator (Table 1). On day 3 the students swab a new plate using the bacterial culture that originated from their initial colony, and again place a blank and antibiotic disk on the plate (Figure 2C). The plates are incubated at 37°C overnight and then placed in the refrigerator. Day 3 of the student lab portion can be completed in approximately 20–30 minutes. After completing the lab exercise, students are introduced to the mechanisms of antibiotic resistance (PowerPoint slides 24–29) and are challenged to draw models explaining how MRSA can become resistant to different classes of antibiotics (Student Handout 3).

On the final day of the lab, students record and analyze their data. Students measure the zone of inhibition (ZOI) surrounding the antibiotic disk for the bacteria plated on day 1 and day 3. Students are provided with detailed instructions on how to measure the ZOI, and a table to record their data (Student Handout 2, p. 5, and PowerPoint slides 30–31). The entire class contributes

Table 1. Timeline of lab exercises and corresponding supplemental material.

	In Class Exercise	Supplemental Material
Pre-Lab Day 1	• Show MRSA video, introduction to antibiotics and prokaryotic cell structure.	• Student Handout 1 • PowerPoint slides 1–14
Day 1 Students	• Students carry out day 1 of the lab exercise. • Discuss the lab setup and make predictions.	• Student Handout 2, pages 1 and 3 • PowerPoint slides 15–22
Day 2 Teachers	• Wrap student plates from day 1 and store in the fridge. • Subculture students' *E. coli* cultures.	• Student Handout 2, page 2 • Guide for Instructors, page 3
Day 3 Students	• Students carry out day 3 of the lab exercise. • Discuss bacterial resistance strategies.	• Student Handout 2, page 2 • Student Handout 3 • PowerPoint slides 23–29
Day 4 Teachers	• Wrap student plates from day 3 and store in the fridge.	• Guide for Instructors, page 3
Day 5 Students	• Data analysis: students observe and record data from their day 1 and day 3 plates. • Compile all class data. • Discuss models. • Students work on their CER arguments supporting either Model 1 or 2.	• Student Handout 2, pages 4–7 • PowerPoint slides 30–33 • PowerPoint slides 34–37 • Guide for Instructors, page 6

their data to a classroom data set, which is used to interpret and evaluate the two models. The students will discuss the models in small groups (PowerPoint slides 30–33), and use information from the entire lab module to fill out the CER sheet to answer the question, "How and why do antibiotics become useless?" (PowerPoint slides 34–37, and Guide for Instructors, p. 6).

As described, this lab was completed for a class that met on a Monday-Wednesday-Friday schedule; however, this lab can readily accommodate other schedules (see Guide for Instructors). For example, the subculture step on day 2 can be continued over multiple days and the plates from days 2 and 4 can be stored in the cold for more than one day before data collection and analysis.

○ Discussing the Models

Our use of prepared models was inspired by the work by Hmelo-Silver, Duncan, and Chinn (2007), who scaffold students' scientific modeling by giving them a set of models to reason with, some of which represent commonly held misconceptions. The two models created for this module are shown in Figure 1. Both models illustrate a starting population of susceptible bacteria that gain antibiotic resistance. Model 1 illustrates needs-based acquisition of antibiotic resistance. Here, the bacteria rapidly gain antibiotic resistance only when exposed to the antibiotic. This model addresses a common misconception that bacteria gain resistance because of antibiotic exposure. Model 2 illustrates acquisition of antibiotic resistance through mutation. Here, the bacteria acquire mutations over time that increase their level of resistance to the antibiotic independent of antibiotic exposure. If antibiotic resistance is acquired early during growth, the population will become resistant. If antibiotic resistance is acquired later during growth, the population may have an intermediate level of resistance.

Using their data, students are asked to consider the fit of each model with the class data. Based on the observation that the starting bacterial populations are susceptible to the antibiotic, despite the antibiotic exposure, students should recognize that Model 1 is inaccurate. Once students reject Model 1, they are challenged to use their data to support Model 2. Since the ZOI indicates that the bacterium is susceptible on day 1, but intermediate or resistant on day 3, students recognize that their data supports Model 2. We allotted time on the final lab day for students to compare individual data to class data, and discuss the models in small groups. The time spent considering their data and the models allows students to recognize that not all the individuals in a population are the same and that a population can gain various random mutations over time.

Finally, students are asked to use the CER approach (Toulmin, 1958) to outline their argument about which model best explains how and why antibiotics become useless (Student Handout 2, pp. 6–7). Students are expected to draw evidence not only from the data acquired in the lab exercise, but also from the introductory material presented throughout the lab module, including that random mutagenesis is required for antibiotic resistance to emerge. Students should argue that both the type and timing of mutation can influence the level of antibiotic resistance. An example of evidence and argument based on student answers related to data from the lab component is provided in the Guide for Instructors (see supplemental materials). We recommend the teacher resource, *Scientific Argumentation in Biology: 30 Classroom Activities* (Sampson & Schleigh, 2013) for additional support in teaching the *NGSS* practice of argumentation.

○ Assessment of the Laboratory Module

This laboratory experience was carried out with nearly 45 pairs of tenth-grade honors biology students across eight classes. Sample

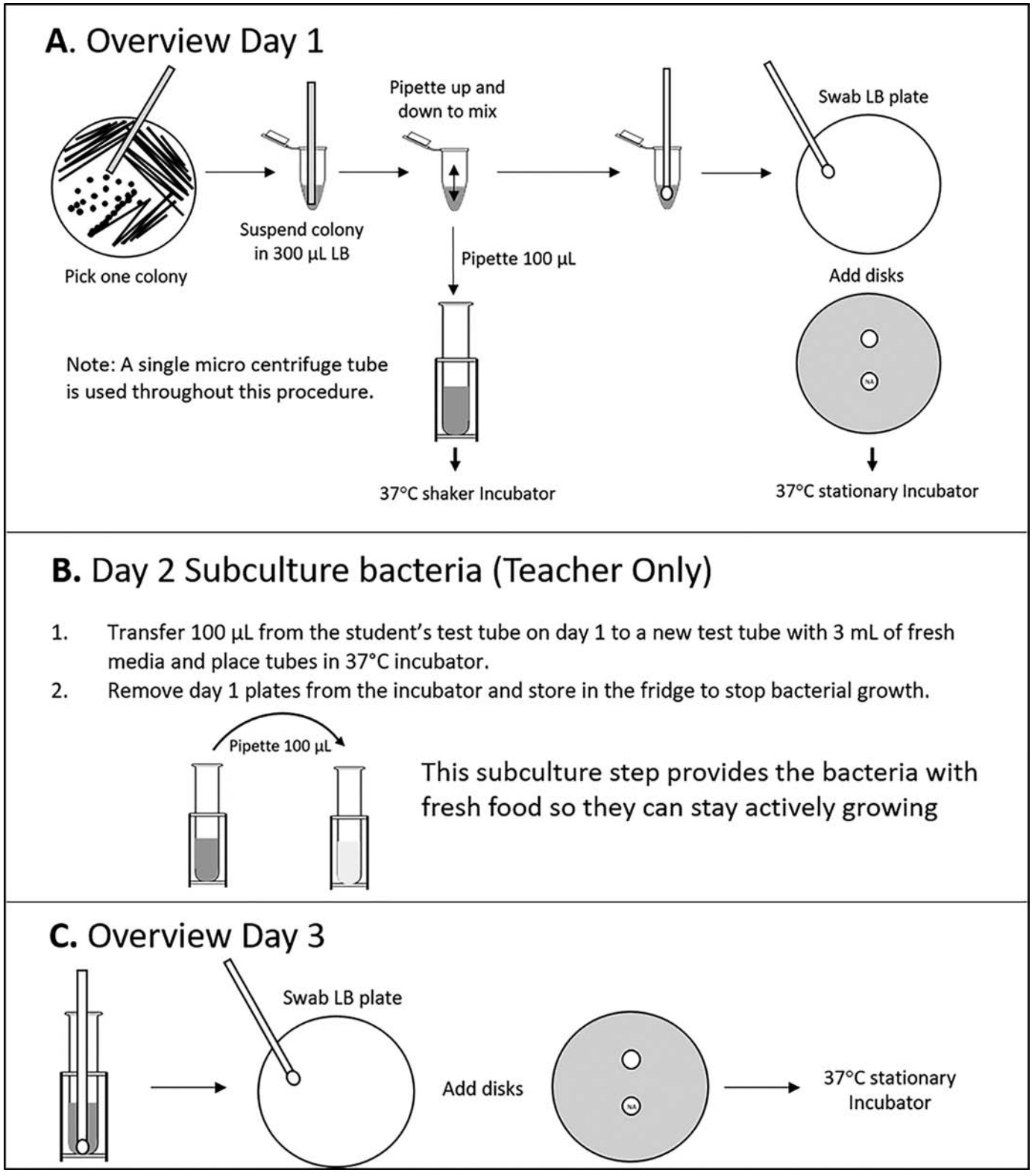

Figure 2. Overview of the lab module.

student plates are shown in Figure 3. Students measured their ZOI and used that number to classify the bacteria as susceptible, intermediate, or resistant per Table 2. The resistance classifications laid out in Table 2 were empirically determined for the conditions tested in this lab exercise and don't necessarily reflect actual numbers used in a clinical setting. Varying the media, temperature, bacteria, or other conditions may change the ZOI classifications.

We pooled the data taken from all classes and calculated the average ZOI to be 31.5 mm on day 1 (Table 2). This indicates that on average, the population of bacteria on day 1 were susceptible to

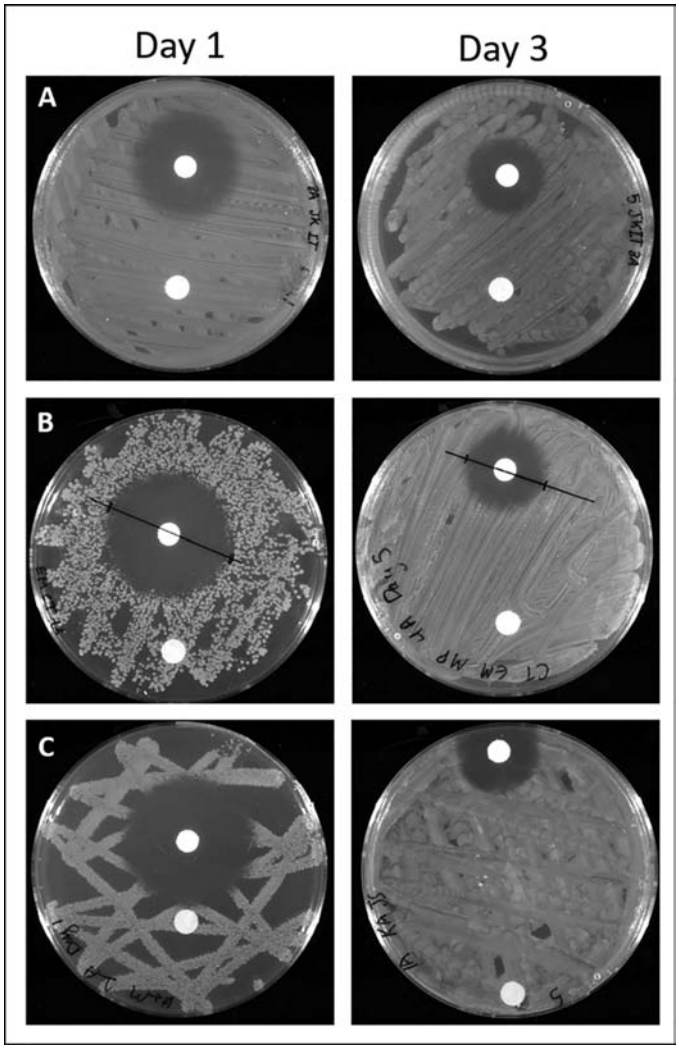

Figure 3. Representative plates from day 1 and day 3 of the lab exercise.

nalidixic acid. Indeed, nearly 93 percent of student pairs had a ZOI that fell into the susceptible range on day 1 (Table 2). Using the class data provides an opportunity to discuss the initial genetic variation within the starting bacterial population. For example, the ZOI ranged from 26 to 44 mm for the susceptible bacteria on day 1 (Table 2).

The diameters of the ZOI observed on day 3 were smaller than those observed on day 1. On average, the diameter of the ZOI was reduced by 10.7 mm. All the student pairs (~93%) that began with susceptible bacteria observed a shift in the classification of their bacteria to either intermediate (~32%) or resistant (61%) (Table 2). Overall, this lab module was easy to execute and provided valuable data to help students learn how antibiotic resistance emerges through mutagenesis.

○ Troubleshooting the Laboratory Module

In implementing this lab module in eight classes, we observed some common problems that were readily corrected with clearer instruction. In this lab, it is ideal to create an even bacterial lawn and appropriately space the disks (see Figure 3A for well-executed sample student plates). In some instances, we observed that the bacterial growth on the day 1 plates was spotted instead of even (Figure 3B, Day 1); however, the edge of the zone was still visible, and students could easily measure the ZOI. In contrast, if the procedure for swabbing the plate was not followed correctly, then the bacterial growth occurred in streaks, which made it harder for proper ZOI measurement (Figure 3C, Day 1). The best procedure for measuring the ZOI was to draw a line through the center of the disk, mark the edges of the zone, and then measure along that line (as illustrated in Figure 3B). In some cases, students placed the disk too close to the edge of the plate (Figure 3C, Day 3). In this case, students can measure the radius of the ZOI and multiply by 2 to get the diameter. Other issues that were infrequently observed included lack of a bacterial lawn, disks that fell off the plate, and contamination. To help prevent or address these issues, we have prepared a section devoted to troubleshooting in the Guide for Instructors.

Table 2. Resistance classification (%) and average ZOI (mm) of compiled student data.

	Day 1	Day 3
Susceptible: ≥ 26 mm	92.9%	7.1%
Intermediate: 25–23 mm	5.4%	32.1%
Resistant: ≤ 22 mm	1.8%	60.7%
Average ZOI	31.5 mm	20.8 mm

○ Supplemental Material

All supplemental materials in this text can be accessed here: http://ri2.missouri.edu/content/Supplemental-Online-Materials. Supplemental materials include student handouts 1–3, a bank of PowerPoint slides, and the Guide for Instructors as referenced in the text. The PowerPoint slide bank also contains additional, optional materials for using a CER format to explain why antibiotics become useless (slides 34–37), for explaining how antibiotic resistance genes on plasmids make antibiotic resistance worse via lateral gene transfer (slides 38–42), and includes videos that demonstrate emergence of antibiotic resistance (slide 43) and summarize key issues regarding antibiotic resistance (slide 44). The Guide for Instructors contains four parts including: materials list, tips for running the laboratory exercise, troubleshooting the laboratory exercise, and an example of how to fill out the CER sheet.

○ Conclusion

Through this lab module, students practice basic microbiology lab techniques and learn key microbiology concepts such as bacterial cell structures and their functions and the mode of action of antibiotics. Students use *NGSS* scientific practices of carrying out an investigation and analyzing data. This laboratory investigation of antibiotic resistance provides an opportunity for teachers to incorporate the high-leverage *NGSS* practices of scientific modeling and

argumentation into high school biology classes. A variety of factors can hamper student understanding of microbiology and evolutionary phenomenon, including: a lack of familiarity with the phenomenon, complicated lab procedures, an abstractness in which the students cannot see what's happening (in this case, individual bacteria are too small to be seen with the naked eye), and/or common misconceptions associated with the phenomenon. In developing this lab and the corresponding models, we included a model that illustrated the common misconception that evolution occurs based on need. Once students considered the needs-based model, they quickly rejected it. By asking students to evaluate the alternative model, we expand their understanding of models, shifting from viewing models only as concrete representations to viewing models as tools for reasoning and predicting biological phenomena. Finally, this lab engages students by applying key concepts in evolutionary biology to a current socio-science topic, leading to discussion of the emergence of antibiotic-resistant bacteria and how we can combat these superbugs.

○ Acknowledgments

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