Sourcing Antibiotic-Resistant *Escherichia coli* in Aquatic Ecosystems: A Combined Laboratory and Field Module

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INTRODUCTION

Widespread use of antibiotics has led to the introduction of low levels of antibiotics spilling over into aquatic ecosystems (1, 2). These residual levels of antibiotics in water sources drive selection pressure for mutations in bacteria that can increase the presence and expression of antibiotic-resistant genes (1, 3, 4). Persistence over time of antibiotic-resistant bacteria in aquatic ecosystems can drive increased risk of infections for humans and animals with resistant pathogens (1, 5), as many freshwater ecosystems are drinking water sources and places for recreation. In addition, prevalence of genes conferring antibiotic resistance in free-living aquatic bacteria may also drive horizontal gene transfer to already problematic or potentially problematic pathogens (2, 5). As a result, the growing emergence of antibiotic-resistant bacteria is of significant concern for public health agents and environmental microbiologists.

Studies examining the occurrence of antibiotic resistance emergence in aquatic environments cannot look for all species of bacteria at once, so they most often utilize common organisms such as *Escherichia coli* that are ubiquitous, easy to work with, and well understood. In addition, *E. coli* is particularly important for freshwater ecosystems for two main reasons. First, *E. coli* is considered an indicator organism, and its presence indicates that conditions may be right for other bacteria or pathogens to persist (2, 6, 7). Second, these bacteria are genetically diverse, so common molecular approaches can be used (e.g., multiplex PCR) to determine what host group or source they may have originated from (8, 9).

As the emergence of antibiotic resistance rises in freshwater ecosystems, it is critical that students realize the potential implications for these ecosystems and for public health. Therefore, student-led research projects that help characterize their local environments for the presence and abundance of antibiotic-resistant bacteria will help highlight the relevance of this growing problem. Using this approach, students gain experience and an understanding of several different fields, including microbiology, environmental science, and molecular biology. In this combined field and laboratory study, students will utilize approaches from multiple disciplines to determine whether environmental isolates of *E. coli* that are sourced from different genetic hosts or different geographic locations can show different patterns in the emergence and presence of antibiotic resistance.

LEARNING GOALS

This learning module can be delivered in a variety of different formats. It can be a standalone module in an environmental science, microbiology, or molecular biology class. It could also be offered as a semester-long research project or larger-scale course-based undergraduate research experience (CURE) or in separate pieces if there are budget or equipment limitations. At the conclusion of this module, students will be able to (i) collect surface water samples from local aquatic ecosystems, (ii) enumerate and culture environmental isolates of *E. coli*, (iii) conduct Kirby-Bauer tests to assess antibiotic resistance in *E. coli* isolates, (iv) use multiplex PCR and gel electrophoresis to identify phylogroups for *E. coli*, and (v) understand that interdisciplinary approaches can be very helpful for answering environmental science, microbiology, and public health-oriented research questions.

PROCEDURE

Students worked in teams to complete the field and laboratory protocol that is provided in Appendix 1. A visual
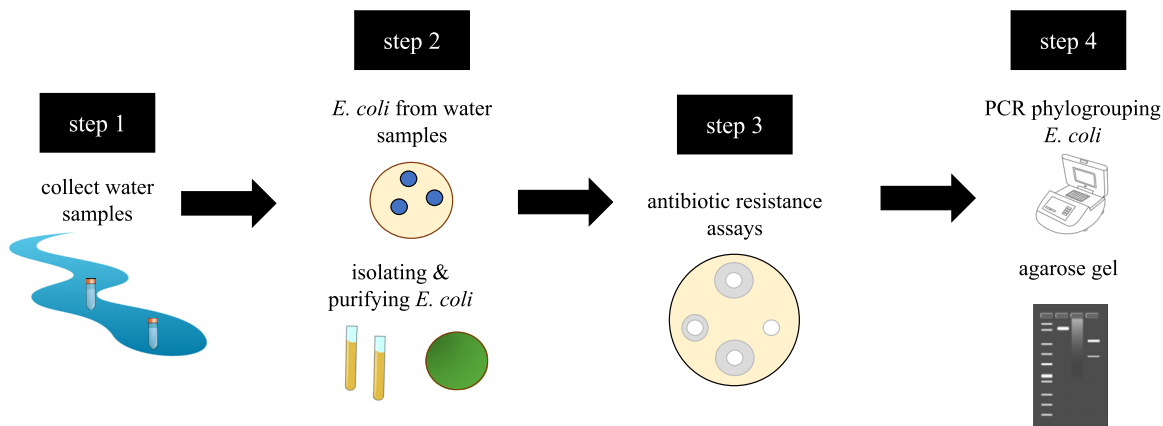
FIG 1. Overview of student activities in learning module on antibiotic resistance in aquatic ecosystems.

guide for the task flow is shown in Figure 1. If completed as a course module, this activity will take 2 to 3 weeks to complete with some work required outside of class time to maintain cultures and complete the PCR work. In total, there are four steps. For step 1, students collected water samples from a local nearby water source that is utilized for either drinking water or recreation. Alternatives could also be places affected by anthropogenic or agricultural sources of waste. Step 2 used water samples collected by students to enumerate and culture *E. coli* isolates. To do this, students used two inexpensive test kits for *E. coli* that are described in Appendix 1: (i) 3M Petrifilm *E. coli*/coliform count plates and (ii) Bluewater Bioscience Coliplate. In step 3, students exposed environmental isolates of *E. coli* to different types of antibiotics using disc diffusion assays, commonly called a Kirby-Bauer test. In step 4, students used multiplex PCR and gel electrophoresis to determine phylogroups for different environmental isolates collected from water samples.

Safety issues

It is also important to note that all work for steps 2 and 3 should be completed in a biosafety level 2 (BSL2) space, as students will be working with unknown microbes that could be potentially pathogenic. This activity assumes that students have had safety training in performing laboratory studies with bacteria. Faculty should look to ASM BSL 2 guidelines for requirements and personal protection recommendations. Students should also know how to dispose of potentially hazardous waste and how to sterilize equipment and surfaces.

CONCLUSION

Students who completed this learning module were assessed for their participation in the project and understanding of issues in environmental microbiology, environmental science, and molecular biology. Specific skills focused on aseptic technique, isolation and culturing of *E. coli*, multiplex PCR, data analysis, and hypothesis/research question development (see

Appendix 1 for potential research questions and potential lecture topics for instructors to help guide student learning). We also included additional qualitative assessment on student confidence levels using a Likert-scale in learning about the intersection of microbiology, environmental science, molecular biology, and public health. Finally, and most importantly, these activities are flexible and designed so that instructors could pick and choose the activities that make the most sense to their courses.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.04 MB.

ACKNOWLEDGMENTS

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