Curriculum
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The PARE Project: A Short Course-Based Research Project for National Surveillance of Antibiotic-Resistant Microbes in Environmental Samples †

Elizabeth A. Genné-Bacon and Carol A. Bascom-Slack*
Center for Translational Science Education, Department of Medical Education, Tufts University School of Medicine, Boston, MA 02111

Course-based research experiences (CREs) have been proposed as an inclusive model to expose all students, including those at institutions without a strong research infrastructure, to research at an early stage. Converting an entire semester-long course can be time consuming for instructors and expensive for institutions, so we have developed a short CRE that can be implemented in a variety of life science course types. The Prevalence of Antibiotic Resistance in the Environment (PARE) project uses common microbiology methods and equipment to engage students in nationwide surveillance of environmental soil samples to document the prevalence of antibiotic-resistant bacteria. The project has been implemented at institutions ranging from community colleges to doctoral-granting institutions in 30 states plus Puerto Rico. Programmatic feedback was obtained from instructors over three iterations, and revisions were made based on this feedback. Student learning was measured by pre/post assessment in a subset of institutions. Outcomes indicate that students made significant gains in the project learning goals.

INTRODUCTION

The benefits of authentic research experiences are well documented (1–8), but these experiences are not accessible to the majority of students (9). Course-based research experiences (CREs) carry many of the same benefits as traditional research (10), yet many instructors struggle to implement this type of course (11). Environmental antibiotic resistance is a subject well-suited for study in a course-based setting: it can be studied using fundamental lab techniques, it provides context to discuss natural selection, and it is broadly relevant and of interest to students.

Antibiotic-resistant infections account for approximately 23,000 deaths per year in the United States (12) and are considered a global threat by the World Health Organization (13). The origin of these infections has remained a subject of intense investigation, but only recently has focus turned to the presence of resistance determinants in environmental reservoirs as a potential contributor to the problem of clinical resistance (14–19). Antibiotics enter the environment through a number of different routes, including discharge from pharmaceutical manufacturing, excretion of unmetabolized compounds used in the treatment of animals and humans, run-off from antibiotics added to animal feed for growth promotion, and discarded, unused medical antibiotics (20–30). The presence of antibiotics in soil and water can provide selective pressure for the enrichment of antibiotic-resistant bacteria and has led to widespread concern about the transfer of resistant organisms to humans through contaminated food or water (15, 31–34).

Better information on the prevalence of antibiotic-resistant organisms in the environment has been suggested as an important step to understand the relationship between environmental and clinical resistance (15), yet this level of tracking is beyond the capabilities of a single research group. The Prevalence of Antibiotic Resistance in the Environment (PARE) project is a crowd-sourcing monitoring system that engages students across the country to systematically test and report the prevalence of tetracycline-resistant bacteria from soil at diverse geographic sites. Subsequent analysis of data collected may provide a preliminary indication of potential hotspots for antibiotic-resistant microbes. In addition, the context of the project provides an excellent example of natural selection. In this paper, we describe the PARE classroom laboratory instructional procedure. This three- to four-class period project uses basic microbiology techniques and is a simple way to engage students in an authentic research project within a classroom setting.

*Corresponding author. Mailing address: Center for Translational Science Education, Department of Medical Education, Tufts University School of Medicine, 136 Harrison Ave., Boston, MA 02111. Phone: 617-636-2479. Fax: 617-636-0375.
E-mail: Carol.bascom_slack@tufts.edu.
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Intended audience

The curriculum is appropriate for, and has been used in, courses in the life sciences, such as biology, environmental science, majors' microbiology, clinical microbiology, and inquiry science courses for nonmajors, assuming students have the appropriate safety training.

Prerequisite student knowledge

Students should be trained in and competent with handling BSL2 organisms. Practice with pipetting and serial dilutions prior to the module results in more accurate student-generated data. This module has no formal curricular/course pre-requisites and has been successfully implemented in nonmajors' biology, introductory biology, and upper-level specialized courses in microbiology and environmental sciences. High school level math skills are required, and knowledge of natural selection is helpful but can be taught in conjunction with the module.

Learning time

Generally, the core PARE module can be implemented over the course of three to four laboratory class sessions (~8 hours total), with the wet-lab portion accomplished in two class periods. Instructors often use 10 to 30 minutes to introduce the project at the end of a previous class period, and students collect soil out of class. The next class period is devoted to serial dilutions and plating of the soil samples, and the following class is spent counting colonies, making calculations, analyzing data, and database entry. If needed and/or desired, instructors can devote a fourth class period to data analysis and presentation (Fig. 1).

- Class 1 (10–30 minutes): Introduce project, instructions for soil collection. Practice serial dilutions if desired.
- Class 2 (2–3 hours): Serial dilutions, plating of soil samples.
- Class 3 (1–2 hours): Count colonies, calculate colony forming units, error-checking.
- Class 3 or 4 (20 minutes): Enter data into the PARE Global Database.
- Class 4: (1–3 hours): Data analysis activities.

Learning objectives

Upon completion of the PARE module, students will be able to:

1. Express and convert numerical values between fractional, decimal, and scientific notation.
2. Calculate the number of colony forming units (CFUs) per gram of soil.
3. Explain the rationale and process for performing serial dilutions on microbiological samples.
4. Explain how antibiotics can provide a selective pressure influencing natural selection of microbial populations.
5. Describe the potential implications for human health posed by the presence of antibiotics in the environment.
6. Represent a given set of authentic ("noisy") data in a table, graph, etc.
7. Reflect on unexpected experimental results and determine the nature of error/troubleshoot.

PROCEDURE

Materials

Materials needed are similar to those used in a typical microbiology teaching lab such as agar plates with media (MacConkey with amphotericin B, with and without

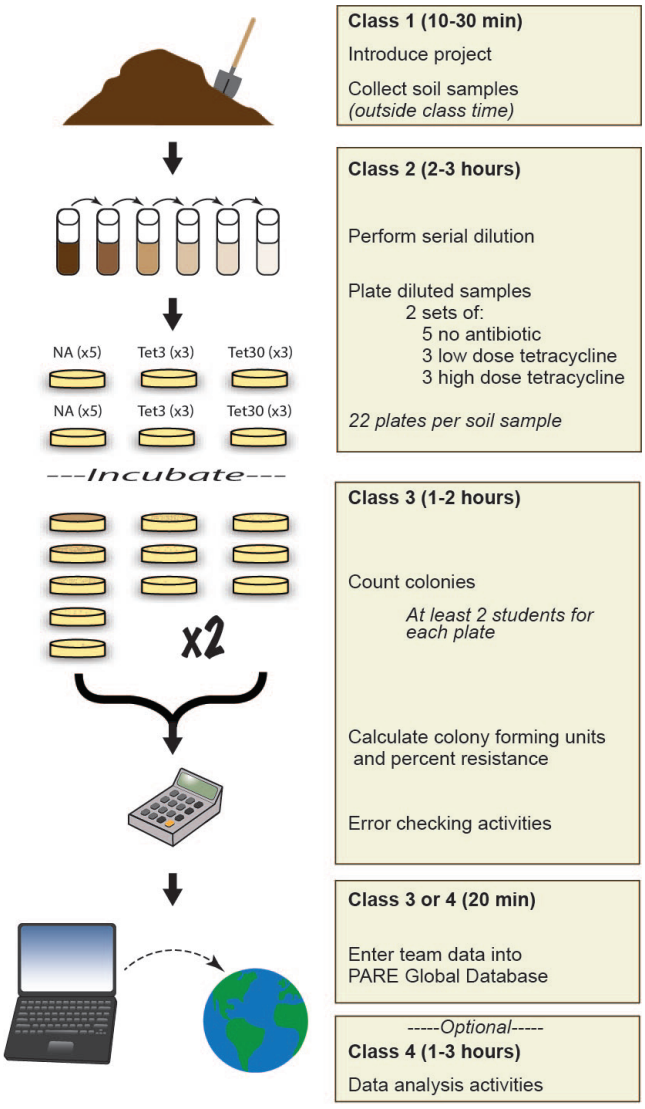

FIGURE 1. Overview of the PARE methods. PARE = prevalence of antibiotic resistance in the environment.

tetracycline), transfer pipettes, analytical scale, screw-cap tubes, spreader, Parafilm, and an incubator. A full list (including suggested alternatives, sources, and product numbers) is available in Appendix 1.

Student instructions

Students are provided a handout that includes background information, worksheets, and protocols (Appendix 1). Briefly, students work in teams of four to collect soil, perform a serial dilution, and plate onto media with and without tetracycline (Fig. 1). Two different levels of tetracycline are used, one of low concentration and one of higher concentration. Students then count CFUs and perform calculations to determine percent resistance. Student-generated data are uploaded into a database after reviewing for accuracy during an in-class activity. In best practice, students form a hypothesis about sources of potential antibiotic resistance and choose soil sample collection locations based on the hypothesis. This can either be done in individual groups, or as a discussion among the whole class. During soil collection, students record collection site characteristics such as GPS coordinates, proximity to a body of water, environment type, proximity to farms, etc. After an opportunity to review results, teams enter all data into the PARE database. Activities for data analysis are suggested.

Faculty instructions

Detailed faculty instructions, including scientific background information, detailed protocols, and other resources, are available in Appendix 1. Instructors are welcome to incorporate the methods used in the PARE project into their classroom without joining the PARE community. However, access to the PARE database requires a 30-minute introductory phone call to review data curation and safety. At this time we also answer questions instructors may have and describe our network collaborative opportunities.

Suggestions for determining student learning

- A pre/post quiz is available to assess student learning outcomes (Appendix 2). Questions are aligned with the learning objectives and provide an objective measure of student learning.
- A practice round of serial dilutions and plating provides an ideal opportunity for students to evaluate their results and consider point-of-error before repeating. Students who have completed a "practice run" serial dilution and plating generally contribute more accurate data. Some PARE instructors have students practice with a sample of known concentration, then use the dilution and plating exercises in PARE as a "lab practical" test of skills.
- During classroom data comparison, instructors might engage students in a discussion about differences observed between groups and within groups. This provides an opportunity to discuss variability, errors, and outliers and the importance of repeating experiments. (For suggestions on how to identify, discuss, and correct errors in student data, see Appendix 1, page 17.)
- Some PARE instructors have opted to require students to make a scientific poster and/or presentation on their PARE research in lieu of a laboratory report.

Sample data

Students record information about the soil sample collection site, colony counts, CFU calculations, and percent resistance. A subset of student data representing two (replicate) plate sets for eight different soil samples is shown in Table 1. CFUs/gram of soil are indicated for the three plate types used (no antibiotic, 3 µg/mL of tetracycline (Tet3), or 30 µg/mL of tetracycline (Tet30)). The resulting percent of colonies resistant to either 3 µg/mL or 30 µg/mL tetracycline are also shown for each plate set.

In first round pilot studies, students plated soil samples onto nutrient agar. Colony enumeration from these plates was challenging due to the diverse colony morphology that resulted (Fig. 2A). In subsequent iterations, we have recommended MacConkey agar for more uniform colony morphology and more consistent colony enumeration (Fig. 2B). Selective agar is often used in environmental studies such as ours (e.g., 35–37).

Safety issues

Student protocols were created to comply with the American Society of Microbiology Guidelines for Biosafety in Teaching Laboratories (38). These guidelines state that culture of environmental unknowns may occur in a BSL1 lab but should be sealed, stored in a secure location, and only observed, not opened or subcultured. Students and instructors are explicitly instructed not to open Parafilm-sealed Petri plates containing cultured organisms. We recommend using BSL2 safety procedures and personal protective equipment since students will be culturing environmental unknowns which could be potential pathogens, and they will be selecting for organisms that are resistant to tetracycline. Tetracycline was chosen, in part, because it is not a front-line antibiotic for treatment of human infections. However, due to the nature of horizontal transfer of resistance determinants, it can be expected that tetracycline-resistant organisms may also harbor resistance to other antibiotics. After observation, plates must be autoclaved prior to disposal. Students should use personal protective equipment, including, but not limited to, safety goggles, lab coats, closed-toed shoes, and gloves. Work surfaces must be disinfected at the end of class and note-taking areas need to be separate from the area where work with microbes occurs. Prior to release of password-protected instructional materials, the safety guidelines are

TABLE 1.
Student data representing a small subset of soil samples.

Collection site characteristics		Plate Set 1					Plate Set 2				
State	Urban, Suburban, or Rural?	CFU No Antibiotic	CFU Tet3	Percent $Tet3^R$	CFU Tet30	Percent $Tet30^R$	CFU No Antibiotic	CFU Tet3	Percent $Tet3^R$	CFU Tet30	Percent $Tet30^R$
OH	Suburban	1.80×10^7	9.40×10^5	5.22	4.50×10^2	0.00	1.65×10^7	9.20×10^5	5.58	5.00×10^2	0.00
TN	Suburban	2.90×10^4	8.30×10^3	28.62	6.20×10^3	21.38	2.80×10^4	6.90×10^3	24.64	5.75×10^3	20.54
TN	Rural	3.75×10^5	1.80×10^3	0.48	TFTC*	0.00	3.65×10^5	1.30×10^3	0.36	TFTC*	0
TX	Urban	4.90×10^6	7.65×10^5	15.61	3.90×10^4	0.80	8.25×10^6	6.50×10^5	7.88	4.50×10^4	0.55
ID	Rural	2.40×10^5	1.45×10^3	0.60	TFTC*	0	2.10×10^5	1.15×10^3	0.55	TFTC*	0
NH	Rural	4.10×10^4	3.40×10^3	8.29	3.00×10^2	0.73	3.4×10^4	4.30×10^3	12.65	5.00×10^1	0.15
KS	Rural	6.35×10^5	1.70×10^5	26.77	6.65×10^4	10.47	7.05×10^5	1.85×10^5	26.24	7.15×10^4	10.14
WA	Urban	1.20×10^5	4.80×10^4	40.00	2.10×10^4	17.50	5.65×10^5	1.20×10^5	21.24	3.55×10^4	6.28

* Indicates that no plate in the plate set has more than 30 colonies, so accurate assessment of CFUs could not be determined.
CFU = colony forming units per gram of soil enumerated from the indicated plate type; Tet3 = MacConkey plates with 3 µg/mL of tetracycline; Tet30 = MacConkey plates with 30 µg/mL of tetracycline; Percent $Tet3^R$ and $Tet30^R$ = the overall percent of colonies per gram of soil that were able to grow in the presence of 3 µg/mL or 30 µg/mL of tetracycline, respectively; *TFTC = too few to count.

emphasized. Instructors are also directed to consult with safety personnel at their institution to ensure that this work conforms to their institutional biosafety requirements.

DISCUSSION

Field testing

The PARE project has been implemented in a variety of course types and in course sizes ranging from 2 to 200 students. As of this writing midway through PARE's fourth year of implementation, a total of 72 undergraduate institutions have participated (20 doctoral, 21 master's, 13 baccalaureate, and 18 associate's institutions). Overall, PARE engages about 2,000 students per year.

Anonymous faculty feedback (via the survey platform Qualtrics) over the first three years of the program resulted in changes to methods and instructional support materials. By the end of the second year, when asked to indicate recommended changes to the instructional materials, comments were favorable overall with no major recommendations for change. When asked to describe what they liked most and least about the program, instructors' comments were generally favorable. Some examples are given below:

> "I liked the fact that it is extremely easy to setup, has a real-world application, and only takes 2 weeks to conduct (4 lab sessions)."

> "Straightforward project that is easy to implement but which has several possible followup activities."

> "The fact that the PARE program takes typical microbiology techniques and applies them to a novel research question. This has allowed my undergraduates to more seriously apply themselves in the lab sessions and work to perfect their microbiology techniques. The data collection can be challenging, but I believe the PARE program has made strides to improve this process."

Evidence of student learning

Pre/post skills survey (objective measure). Student learning was assessed using a pre/post test aligned with the PARE learning goals that contains 13 questions, including multiple choice, free response, and data interpretation. (The list of questions, as well as the grading rubric and target learning objectives, is available in Appendix 2.) Prior to administering the test to students, instrument feedback was obtained from a subset of PARE instructors regarding clarity of the questions and anticipated difficulty level for their students. The test has been administered in a few PARE classrooms. Results described here are from students at five different institutions: one STEM-focused master's granting university, one community

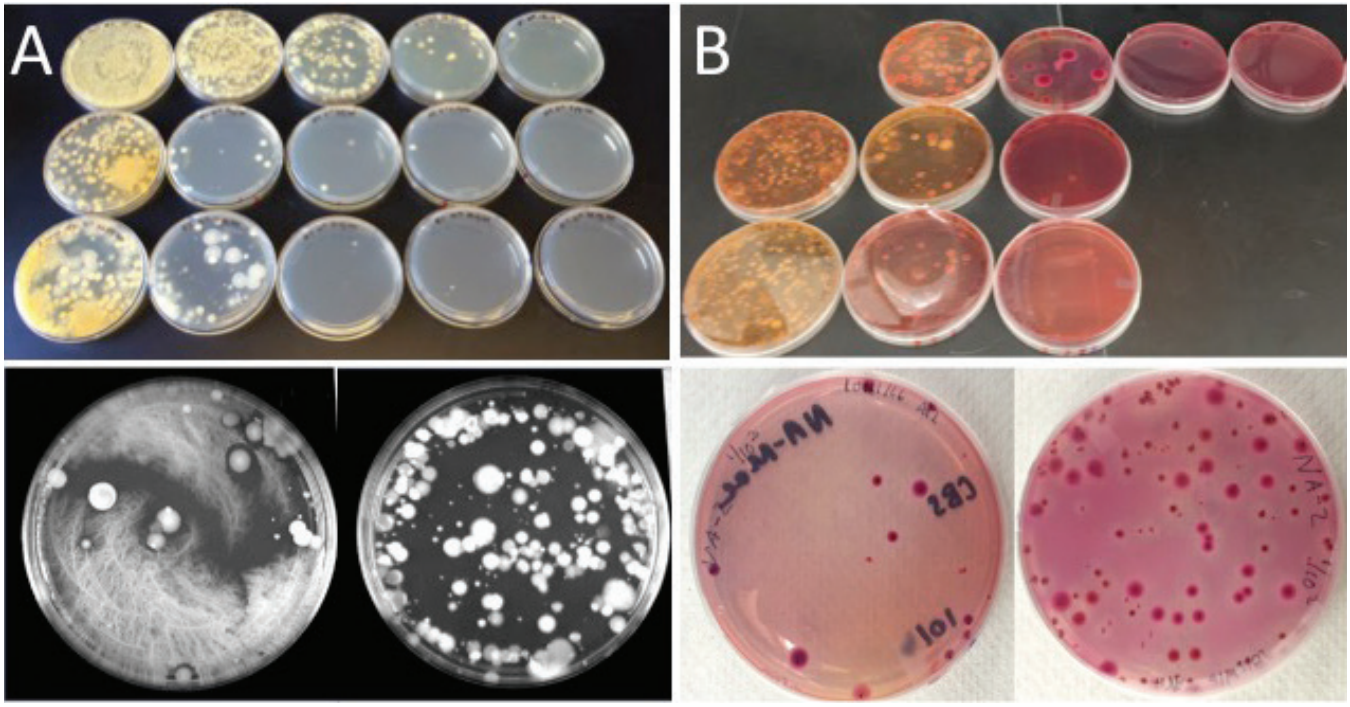

FIGURE 2. Sample data—serial dilution plating sets. A) Nutrient agar. The top photo shows the entire plating series. The bottom images show individual plates with diverse colony morphology rendering accurate enumeration difficult. B) MacConkey agar. The bottom images show the more uniform colony morphology of MacConkey agar.

college, one primarily undergraduate university, and one large public research university. The test was conducted anonymously online using Qualtrics. Students were given the pre test prior to the implementation of any PARE-related activities. The post test (identical to the pre test) was administered after all PARE-related activities had ceased. Open-ended responses were scored by a researcher blinded to the pre/post status of the entries, using a points-based rubric. Incomplete responses and responses with no corresponding pre or post tests were eliminated from analysis.

The average pre test score was 58% correct (a score of 7.74 out of 13.25 possible points). After participating in the PARE project, post test scores rose to an average of 71% correct (9.47 out of 13.25 possible points). A paired *t*-test of matched pre and post test scores shows that this increase is highly statistically significant ($p < 0.0001$, $n = 43$), with an effect size of 0.83 (Cohen's *d* repeated measures, pooled standard deviation), indicating a strong effect of the PARE project on student learning gains as measured by our test (Fig. 3).

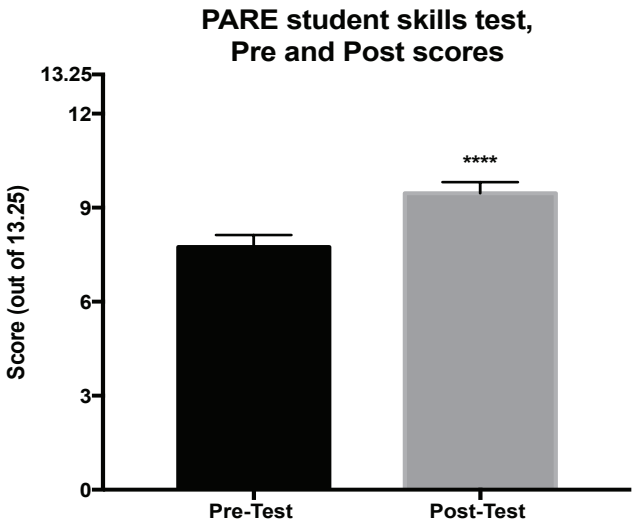

FIGURE 3. Student learning after the PARE module. Bars represent average scores with standard mean error. Pre and post tests are identical, and scores are out of 13.25 possible points. A paired *t*-test reveals a significant ($p < 0.0001$) effect of the PARE experience on post test scores. $n = 43$, **** $p < 0.0001$. PARE = prevalence of antibiotic resistance in the environment.

Student evaluations (subjective measure). At the conclusion of PARE, students fill out a feedback survey (Qualtrics). Responses to open-ended feedback questions indicate that PARE has provided insight to possible, previously unknown, career possibilities. For example:

> "Now that I know what it is like to work in a true lab setting, I may want to work in a lab for a career."

> "I never enjoyed lab and research before, but I have genuinely enjoyed microbiology lab, and this has lead me to be open to possible future work in this field."

Alternatively, for some, the experience confirmed their interest in research:

> "Doing the research helped out a lot and confirmed that this is something that I would love to do. I'm wanting to go into research work with marine environment. This experience just confirmed that it is something I would love to do."

Possible modifications

Data contributed to the national database must be obtained using systematic methods, but beyond that, instructors have reported that they like the flexibility of the module in terms of ease of adaptation to a particular course and in terms of follow-up expansion possibilities. Many instructors have used the PARE module as a starting point to launch a full semester or extended CRE with additional activities such as statistical analysis of the classroom or group dataset and molecular amplification of 16S rDNA to assess phylogenetic placement of individual colonies.

SUPPLEMENTAL MATERIALS

Appendix 1: Course materials
Appendix 2: PARE student pre-post assessment

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