

A Large-Class Undergraduate Microbiology Laboratory Activity on Microbial Diversity and Antimicrobial Resistance

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The investigation of microbial diversity and adaptation is essential to comprehend biological processes. Yet, teaching basic microbiology techniques to large groups of students in limited time is challenging, as most approaches are time-consuming or require special equipment. In this activity, students performed three laboratory exercises in three hours involving the analysis of inoculated agar plates they prepared by swabbing samples from an environment of their choice, the examination of antimicrobial effects on growth, and the assessment of microbial enzymatic activity in soil. The activity was field tested in two classes (70 and 76 students, respectively) of first-year undergraduate biology and zoology students at the Bangor University (UK) using pre- and post-tests ($n = 84$). Based on the answers, learning gain scores (G) were calculated for each learning objective (LO). For all LOs, the mean post-test scores were higher than the mean pre-test scores. The activity significantly improved students' understanding of microbial diversity ($G = 0.36$, $p = 0.010$) and microbial detection and quantification ($G = 0.18$ to 0.773 , $p \leq 0.004$). The lack of significant differences in scores for questions targeting microbial growth ($G = 0.31$, $p = 0.292$) and antimicrobial resistance ($G = 0.38$, $p = 0.052$) suggested some existing knowledge amongst undergraduates. However, the extent of knowledge showed great variation. The results may suggest that the activity is suitable to introduce microbiology-related laboratory work to students with limited laboratory skills and knowledge. Furthermore, the pre- and post-test approach used here is suitable for both course evaluation and monitoring attainment and can be used for program validation and quality control.

INTRODUCTION

Microorganisms form a diverse group of microscopic organisms existing as single cells or colonies, found in great numbers across the biosphere, and represent approximately 60% of the global biomass (1). They play a crucial role in the foundation of our biosphere and in biogeochemical cycling, and through their diverse metabolic adaptations, they offer huge medical and biotechnological potential (2). Microorganisms, especially prokaryotes, show high physiological and biochemical versatility and they are able to adapt to the most extreme environments. Therefore, the examination and description of the microbes found in different environments are crucial to understand the major biological processes in our biosphere.

There are many approaches to describe the microbial ecology in the environment. The traditional method is isolation and cultivation, which requires days of incubation. Other, less time-consuming approaches may require specialized equipment (e.g., microscopes, thermocycler, spectrometer) or prior knowledge and skills in microbiology. As student numbers are increasing and resources are limited, these methods may not be suitable for large classes of students who have limited time for practical work. Consequently, laboratory exercises are often replaced with complementary activities, such as literature searches or data interpretation and analyses (3). Nonetheless, the skills and practical understanding of the fundamentals of microbiology are still essential in all undergraduate biology programs (4).

This curriculum describes a short, three-hour activity which involves skill development in some fundamental microbiological methods: agar plating for isolation, colony counting, recording of colony form and a soil catalase assay. The activity provides students with hands-on experience and skills to study microbial diversity, activity, and antimicrobial resistance. They learn basic microbial quantification techniques as well as microbiology laboratory health and safety rules. Usually, these activities are covered with multi-week

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laboratory exercises (5, 6). However, that may not be an option for large undergraduate classes.

Intended audience

This laboratory activity has been used in an undergraduate microbiology course designed for first-year biology and zoology students. The activity is suitable for students who have no previous experience in microbiological laboratory work. The activity is suitable for classes with up to 100 students.

Learning time

The activity described here takes three hours to complete (Table 1). The first part is a 30-minute sampling while students take swab samples from their environment and transfer the sample to agar plates (Appendix 1). The sampling kits, along with instructions, are distributed during a lecture at least three days prior to the activity, and samples are incubated for microbial growth prior to the practical. The second part is a laboratory activity that consists of a short introduction (Appendix 2) followed by three exercises (Appendices 3 and 4); each exercise takes approximately 40 minutes to accomplish. The first exercise focuses on microbial diversity, the second on antimicrobial resistance, and the third on microbial activity.

Prerequisite student knowledge

Prior to the activity, the students should have attended general microbiology and microbial diversity lectures covering the structure, function, reproduction, genetic recombination, and evolution of bacteria, archaea, fungi, and viruses. However, a basic understanding of microbiology is sufficient for attending this class. Hence it can be scheduled at the beginning of the semester.

Learning objectives (LOs)

By the end of this activity students should be able to:

1. Compare microbial communities from different environments
2. Identify factors that affect microbial growth
3. Isolate microorganisms on agar plates
4. Describe the appearance of, and quantify, microbial colonies
5. Explain the mechanisms and effects of antimicrobials
6. Perform a catalase assay to measure microbial activity in soil

TABLE 1.
Brief description and timeline of activities.

Activity	Duration	Performed by
Sampling 3–6 days prior to laboratory activity		
Collection of swab samples Spreading of swab on two agar plates	30 min	Unsupervised students
Addition of antimicrobials to one of the agar plates	1–3 h	Faculty/instructors
Incubation	2–6 days	
Laboratory activity		
Introduction	30 min	Faculty/instructors
Exercise 1: examination of agar plates	40 min	Students in groups supervised by faculty/instructors
Exercise 2: examination of agar plates with antimicrobials	40 min	Students in groups supervised by faculty/instructors
Exercise 3: catalase activity assay on soil samples	40 min	Students in groups supervised by faculty/instructors

PROCEDURES

Materials

Materials and equipment for one class of 100 students working in groups of five for the different activities are listed here.

For sampling and sample incubation:

- 100 sampling kits containing a single cotton swab and two nutrient agar plates with adhesive labels in a resealable plastic bag.
- 10 mg/mL ampicillin solution, 1 mL
- Garlic (one fresh bulb or 10 g powder)
- Ginger (one fresh root, or 10 g powder)
- Cinnamon (two sticks or 10 g powder)
- Coconut oil, 10 mL

For laboratory activities:

- 100 markers used for labeling and counting colonies in Exercise 1
- 20 calculators for counting colonies in Exercise 1
- 100 rulers for measuring colony size (Exercise 1) and area affected by antimicrobials (Exercise 2)
- 3 different types of air dried and sieved soil samples, 250 g each
- 80 50-mL centrifuge tubes. Three tubes are used for the soil samples and one for the water in Exercise 3
- Approx. 120 squares of parafilm (5 × 5 cm) for sealing centrifuge tubes
- 20 pieces 20-cm plastic tubing (inner diameter 4–5 mm) to inject hydrogen peroxide into soil and connect the tube with soil to the one with water
- 20 10-mL syringes
- 20 60-mL 3% hydrogen peroxide solution. A 10-mL aliquot is added to each sample.
- 5 spray bottles of 70% ethanol (or equivalent) solution for removing marking from agar plates in Exercise 1 and 2.

Student instructions

Three to six days prior to the activity (during a lecture), the sampling kits are distributed to the students (Table 1). Students are instructed by the lecturer on the use of the swab and how to distribute the swab sample on the agar plates to create a streak plate (Appendix 1). Students are encouraged to take swab samples from surfaces they believe have a diverse microbial population (e.g., pond, soil, food waste container). Taking human samples (skin, saliva, other excreta) is not allowed. The samples are then taken to faculty member(s) within 24 hours of sampling (two to six days prior to the laboratory activities).

Directions for instructors

Once the nutrient agar plates with samples are received, one set of the plates serves as controls and the others

are treated with a selected antimicrobial compound. One antibiotic is a penicillin-derivate (ampicillin) and the rest are “natural antimicrobials,” natural substances that are believed to cure infections. However, their usefulness is not well studied (8–11). In this example, we used substances that occurred first in a Google search (performed by staff while planning the activity) for “natural antibiotics” and were available in local supermarkets (Appendix 2, slides 10 and 11). We used garlic, ginger, cinnamon, and coconut oil.

This exercise is specifically designed for large classes of 50 to 100 students with very little or no experience working in microbiology laboratories. Hence, the main aim of the activities is to introduce laboratory work to the students and show them examples of microbial diversity and antimicrobial resistance they learned about in their lectures. Instructors (four staff members for 100 students) are asked to circle the laboratory while the students are performing exercises to help and to ask questions regarding the outcomes.

The faculty member leading the activities should re-emphasize the health and safety instructions at the beginning of the laboratory work and give a brief introduction on the theoretical background of the exercises, including methods for detecting bacteria, genetic recombination and the role of the catalase enzyme, before each exercise (example can be found in Appendix 2). Each exercise is followed by a group discussion led by a faculty member to answer the questions detailed at the end of each exercise in the handout (Appendix 3).

Detailed preparation instructions

One day before distributing sampling kits. For 100 students, prepare 200 nutrient agar plates and assemble sampling kits containing two nutrient agar plates and one cotton swab in a resealable plastic bag.

On the day of sampling. Ideally samples are taken to a faculty member or teaching assistant by early afternoon. One set of the duplicated plates serves as the controls (no treatment, labeled as ‘C’), and the other set is treated with antimicrobials. The five types of antimicrobials (ampicillin, garlic, ginger, cinnamon, and coconut oil) are randomly assigned. Place the antimicrobials in the center of the plate and label “Amp,” “Ga,” “Gi,” “Ci,” or “Co.” Use 10 μ L of the ampicillin and the coconut oil and 0.5 × 0.5-cm slices of the garlic, ginger, and cinnamon. When powder is used, it should cover a 1 cm² area of the plate. Incubate all plates at room temperature (20 to 22°C) for three days and seal them with parafilm. If the sampling is completed more than three days before the laboratory activity, store plates at +4°C until they are needed. Sieve and air-dry soil samples for Exercise 3.

The day of the laboratory activities. Enable students to collect their own plates. Supply each laboratory station, accommodating a group of up to five students, with four centrifuge tubes, one section of plastic tubing, parafilm, a ruler, a calculator, and markers. At the beginning of the

activity, students should receive the Student Laboratory Handbook (Appendix 3) and a risk assessment form.

Summary of laboratory exercises

Exercise 1. The purpose of this exercise is to familiarize students with microbial colony morphology and appearance. Students are expected to describe the size, shape, color and elevation of the colonies they find on their control plates. Working in groups, they should examine five different plates, preferably with samples taken in different environments (Fig. 1). Students should also count the number of colonies and rank the samples based on microbial diversity (i.e., number of colony types) and abundance (i.e., absolute number of colonies). At the end of the exercise, the reasons for low/high abundance are discussed with the help of the instructors. Students should not open their plates during the exercise and wipe the markings using 70% ethanol (or equivalent) before swapping plates.

Exercise 2. This exercise shows examples of the effects of antimicrobials. The students examine plates treated with different antimicrobials and rank the effectiveness of the antimicrobials based on the size of the affected area and number of colonies in the affected area (Fig. 2). Students are encouraged to swap plates to compare different antimicrobials in different environments. The effects of antimicrobials are not directly comparable, but the results should enable students to understand the effects of antimicrobials and should promote discussion. At the end of the exercise, the reasons for differences in the effectiveness of antimicrobials are discussed with the help of the instructors. Students should not open their plates during the exercise

and should wipe the markings using 70% ethanol (or equivalent) before swapping plates.

Exercise 3. This exercise is adapted from Toth et al. (12) and focuses on microbial activities in different soils. During the exercise, catalase production is measured as a proxy for microbial activity. Catalase is an enzyme produced by most aerobic bacteria, and its role is to break down hydrogen peroxide (by-product of metabolism) to water and oxygen. To measure catalase production, contrasting soil samples (in terms of organic content) are mixed with hydrogen peroxide and the oxygen produced by catalase is transmitted to water using a plastic tube (Fig. 3) so the bubbles produced can be counted. The soil samples should be ranked based on their catalase activity, which refers to microbial activity.

Determining student learning

This activity was not formally assessed, and performance was not incorporated into students' grades. The usefulness of the activity was tested using the pre- and post-test approach described in Allen and Gyure (5). Students were asked to complete a short test of seven questions, targeting the LOs, before and after the laboratory activities. The test with the instructors' key is in Appendix 5. In this study, 84 paired pre- and post-tests were evaluated and rated according to the rubric. As two questions (Q3 and Q4) addressed LO3, those were treated as one in the rubric (Appendix 6). Two-tailed *t*-tests were used to indicate whether the difference between corresponding pre- and post-test scores was significant ($p \leq 0.01$). The learning gain (G) on a scale of 1 to 4 was calculated for each LO as: $G = (\text{post-test score} - \text{pre-test score}) / (4 - \text{pre-test score})$.

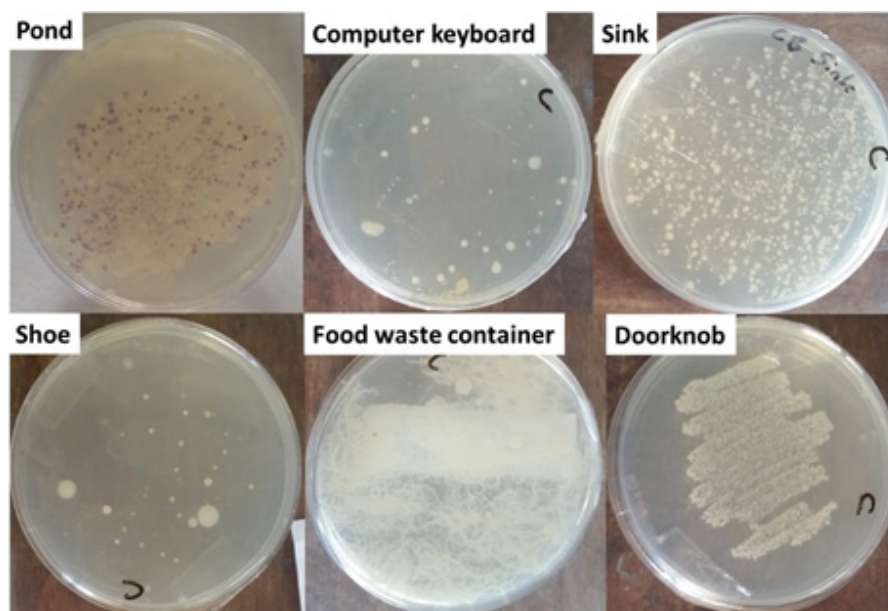


FIGURE 1. Examples of plates used in Exercise 1.

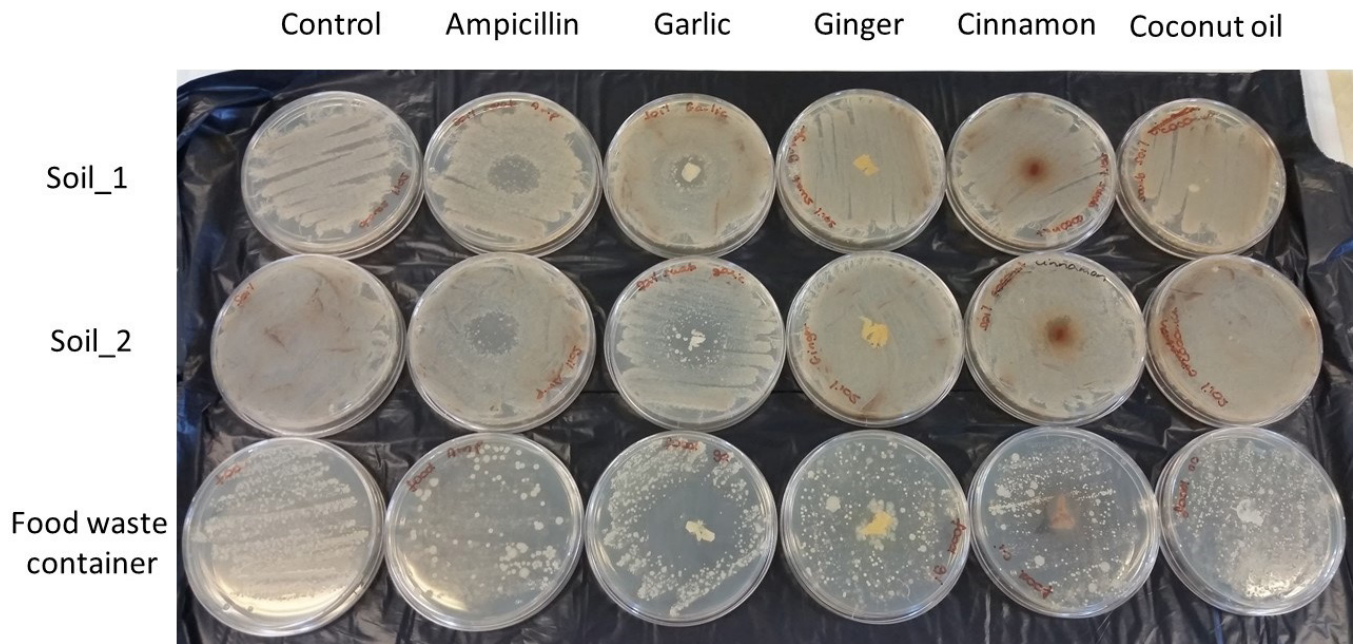


FIGURE 2. Examples of the effects of antimicrobials.

Safety issues

A risk assessment form in accordance with the university's health and safety regulations should be prepared and circulated among students prior to the activity. During the laboratory work, general microbiological laboratory health and safety rules are applied in compliance with the American Society of Microbiology Guidelines for Biosafety (7). In accordance with good laboratory practice guidelines, the work may be carried out in a biosafety level 1 (BSL1)

laboratory, as the plates containing unknown environmental strains are sealed with parafilm and remain unopened during the exercises. The plates are autoclaved after the activities. Students and staff members wear appropriate personal protection equipment (laboratory coats and closed-toed shoes) in the laboratory and remove the coat and wash their hands before leaving the laboratory for any reasons. Long hair should be tied back during laboratory work. Students and staff members should wear nitrile gloves while handling 70% ethanol (or equivalent) and during Exercise 3. Work surfaces should be disinfected prior to and after the exercises.



FIGURE 3. Set-up for testing microbial catalase activity in soil (Exercise 3). Tube 1: soil mixed with hydrogen peroxide solution. Tube 2: 30–40 mL water.

RESULTS AND DISCUSSION

Student engagement and feedback

The main goal of this activity is to engage students with microbial diversity and function in the environment, illuminate the current challenges with antimicrobial resistance, and familiarize the students with basic laboratory procedures to test some of these concepts in a stress-free environment. The activity aimed to follow the principles of the MUSIC® Model of Motivation (13): ensuring that students feel empowered (having control over their studies); they find the activity is useful; they can succeed; they find the material interesting; and the instructors care about their well-being (14). The use of everyday household products that students are familiar with as antimicrobial compounds and the aim of assessing the reliability of information derived from the Internet increase curiosity and engagement. The students are encouraged to collect their own samples from an environment of interest and hence take owner-

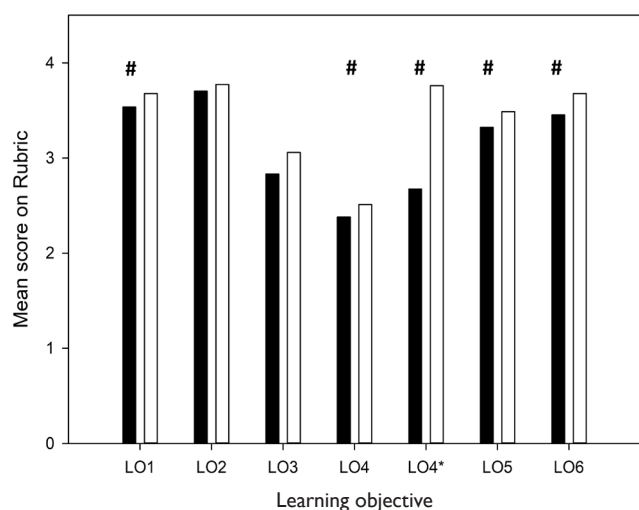


FIGURE 4. Rubric scores for the four learning outcomes. Mean ($n = 84$) student scores on a rubric (Appendix 5) were used to measure knowledge of the four LOs before and after the class (i.e., pre- and post-test, shown as black and white bars, respectively). *, unanswered questions were excluded ($n[\text{LO4}^*]=46$); #, differences between pre- and post-test mean scores are significant ($p \leq 0.01$).

ship of their learning, which is more engaging than working with samples collected or prepared by staff and fosters a student-centered approach to learning. During this laboratory activity, students work in small groups to discuss their findings and debate their conclusions, with ad hoc input from teaching staff. Learning in small groups is favored by students and has been shown to promote academic achievement and effective learning in many fields of biology (15–18). It also influences the climate of the classroom providing a relaxed environment where students are more likely to contribute (19). However, the benefits of group learning may be shadowed by “passengers” (i.e., unmotivated students making little contribution) (20). Therefore, tutors move amongst the groups during each exercise to encourage all students to participate. Group learning and student engagement with the subject and a relatively stress-free environment with appropriate workload have been shown to promote deep learning (21, 22), which should be encouraged at the university level (22, 23).

Student feedback suggested that the sessions provided a relaxed environment for learning basic microbiological methods and were suitable for a diverse group with very different backgrounds. Feedback included the following comments: “Interesting and not hard to follow”; “A very engaging practical, it was interesting to see different samples with unexpected results”; “Interesting revision of microbial techniques.” However, some comments suggested that Exercise 3 caused some trouble as the tubes were hard to seal with parafilm (“Bungs preferable. Had a bit of an issue with bubbles/sealing tube.”). Therefore, the application of parafilm needs to be explained in the future. Staff members involved in the class also found it a positive experience

(“the class really enjoyed the practical and the results were great! It was interesting for demonstrators as well to see what had been sampled and what the antimicrobials had affected. Overall it was an interesting practical, which fully engaged the class.”).

Field testing

This curriculum exercise has been used for two academic years in an undergraduate microbiology unit designed for first-year undergraduate biology and zoology students as part of the “Principles of life” first-year undergraduate module at the Bangor University, UK. This curriculum was field tested in spring 2018 in two consecutive classes (morning and afternoon sessions). In total, 146 students attended the two classes (70 students in the morning and 76 students in the afternoon). Students were asked to take a test before and immediately after they finished the activity. The tests were voluntarily taken by 21 and 63 students attending a morning and an afternoon session, respectively. The field tests showed that the time was sufficient to perform activities and no health and safety issues were raised.

Evidence of student learning

Student learning was monitored using pre- and post-tests to measure learning gain of each LO. The rubric scores were calculated based on the answers the students gave in the tests addressing the LOs before and after the activity. We assessed whether the difference between scores calculated for the pre- and post-tests were significant and quantified learning progress by calculating G scores, as described in the Determining student learning section.

For all LOs, the mean rubric scores were higher for the post-tests than for the pre-tests (Fig. 4), suggesting that the activities successfully addressed the LOs. Learning objectives 1 and 2 were assessed in questions 1 and 2, respectively. Answers to both questions showed an increase in rubric scores, suggesting learning gain. However, the increase was not significant for question 2 (Table 2). The lack of significant differences in mean scores between pre- and post-tests for LO2 suggests that the students had sufficient previous knowledge on the subject. This is further supported by the fact that 81% of the students scored 4 answering question 2 in both pre- and post-tests.

The G score for LO3 (questions 3 and 4) was low (Table 2). Only 15% of the students answered the questions correctly before and after the exercises and the outcomes suggest that the students had no extensive knowledge and experience on the subject. Therefore, in the future these aspects should be better explained during the activities.

Interestingly, no notable gain was observed for LO4. Question 5, addressing LO4, was the most difficult and time consuming and hence many students (45%) did not spare the time to answer that question. After eliminating the tests where question 5 was not answered, significant

gain was observed (Table 2 and Fig. 4), suggesting that the exercise successfully addressed the method of microbial colony counting. However, the lack of answers to question 5 may indicate that the question was too difficult to answer, even after the activities. This would imply that while some students understood the concept of colony counting, others may have struggled and hence the quantification of microbial colonies should be better explained in the future.

High scores were observed for LO5 and LO6 (Table 2), addressing antimicrobial resistance and the measurement of microbial activity in soil, assessed in question 5 and question 6, respectively. Overall, the results suggest that LOs 1 and 2 and LOs 5 and 6 addressed novel aspects of microbiology for the students and that the information was well-addressed during the exercise. In order to maximize student learning, LO3 and LO4 should be better addressed and explained in the future.

The G scores were also calculated separately for the students attending the morning and afternoon sessions (Table 2). For most LOs, higher G scores were observed for the morning session than for the afternoon class. That may be the result of fatigue during the afternoon session. Nonetheless, the results suggest that the questions on antimicrobial resistance and the detection of microbial activity were addressed slightly better during the afternoon session than in the morning. That may be a result of the questions raised during the morning class. As the demonstrators answered many questions regarding these topics in the morning, those points were better explained during the afternoon session.

Possible modifications

The main aim of this activity is to enable students to engage with basic, cultivation-based procedures in a very limited time. The main activities associated with this exercise are performed in one three-hour session. Nonetheless, the activities could be split into two sessions three to seven days apart. The first, one-hour session would consist of an introduction, soil collection and preparation, sample collection, plate preparation, and antimicrobial selection, during which the students would be encouraged to select sampling areas and antimicrobials. The second two-hour session would be sufficient to perform exercises 1 to 3 and discuss the results. When sampling prior to the laboratory activity is not feasible, technical staff members can prepare plates (one pair of plates for five students). The activity can also be extended to a multi-week project. During the second session, students isolate a bacteria colony from their own plate and try to maintain them under different conditions (24). Using various approaches (e.g., Gram staining, Voges-Proskauer reaction, Hugh-Leifson test, polymerase chain reaction [12]), the strain can be determined. In that case, the exercises should be conducted in a BSL2 lab in compliance with the American Society of Microbiology Guidelines for Biosafety (7).

Based on the results of the pre- and post-tests, the students were familiar with the theoretical aspects of microbial detection and antimicrobial resistance. Therefore, for the students enrolled in this program, explanation on these subjects were not necessary during the session. Nonethe-

TABLE 2.
Mean student learning gains (G) for each learning objective

Learning objective (questions used to test LO)	G score (p)		
	All students (n=84)	Morning class (n=21)	Afternoon class (n=63)
1. Compare microbial communities from different environments (1)	0.36 (0.010)	0.55 (0.055)	0.25 (0.083)
2. Identify factors that affect microbial growth (2)	0.31 (0.292)	0.43 (0.104)	0.22 (0.698)
3. Isolate microorganisms on agar plates and assess microbial cultures (3, 4)	0.18 (0.001)	0.21 (0.162)	0.17 (0.017)
4. Describe the appearance of, and quantify, microbial colonies (5)	0 (0.434) 0.77 (<0.001)*	0.30 (0.188) 0.88 (0.007)*	0 (0.933) 0.73 (<0.001)*
5. Explain the mechanisms and effects of antimicrobials (6)	0.38 (0.052)	0.17 (0.428)	0.46 (0.077)
6. Perform a catalase assay to measure microbial activity in soil (7)	0.63 (0.004)	0.44 (0.016)	0.74 (0.067)

P values (based on two-tailed t-tests) indicate whether the differences in G scores calculated for pre- and post-tests are significant. *: unanswered questions were excluded (n[LO4*] was 13 and 33 during the morning and afternoon classes, respectively).

less, when the session is attended by students with different backgrounds, their knowledge of these subjects should be examined prior to the activity.

In order to reduce the impact of the activity on the environment, glass consumables instead of plastics should be used. Note that this would increase the time required for cleaning up at the end of the session and/or the involvement of technical staff after the session. To reduce the time required for preparation, ready-to-use nutrient agar plates can be purchased and used.

CONCLUSION

In this paper, a three-hour activity is described aiming to engage first-year undergraduate students with basic microbiological approaches and to familiarize them with laboratory health and safety. The session is designed as a first, introductory class for students with no or very limited laboratory experience. The activity is suitable for a diverse group of students with differing knowledge of microbiology.

The pre-/post-test approach coupled with rubric analysis is suitable for the evaluation of the session. The method enables a rapid assessment of the strengths and weaknesses of the activities without pressuring students and teaching staff. By addressing the issues raised by the test, the activity can be easily modified to better fit students' needs.

SUPPLEMENTAL MATERIALS

- Appendix 1: Instruction slides for sampling
- Appendix 2: Introduction and instruction slides for the group discussion of the results
- Appendix 3: Student laboratory handbook (protocols and worksheets): Microbes and antimicrobials
- Appendix 4: Demonstrator's copy of laboratory handbook (protocols and worksheets): Microbes and antimicrobials (including instructor's key)
- Appendix 5: Pre- and post-test: Microbes and antimicrobials (including instructor's key)
- Appendix 6: Grading rubric for pre- and post-test

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