

Introducing First-Year Undergraduate Students the Fundamentals of Antibiotic Sensitivity Testing through a Combined Computer Simulation and Face-to-Face Laboratory Session

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Determining the antibiotic sensitivity of disease-causing microorganisms is a fundamental process in a clinical microbiology laboratory. With the continued use of antibiotics, the emergence of antibiotic resistance has become a significant health issue. However, the principles and laboratory testing to determine antibiotic sensitivity are generally not taught to first-year undergraduate students. This is partly due to the limited time to cover the fundamental biology of microorganisms and the mechanism of action of antibiotics in an introductory course. We overcame these limitations by teaching first-year students the fundamental principles of antibiotic sensitivity using an online data generator/simulation. Using the Kirby-Bauer disk diffusion test, students replicated the effects of antibiotic dose on bacterial growth and determined the antimicrobial susceptibility testing of their allocated bacterium. After 2–3 weeks, the antimicrobial sensitivity testing was replicated in an authentic face-to-face laboratory setting over 2 days. The impact of the intervention on student learning was assessed using a written laboratory report and a short questionnaire containing Likert and free-text questions. Student self-reported understanding of the content rose significantly, with nearly all students passing the written assessment. The approach was found to be enjoyable and interactive and facilitated authentic learning in first-year students. This cohort of students will continue to use more advanced versions of this simulation in future years, allowing for the long-term benefits of this approach to be assessed.

KEYWORDS hybrid teaching, antibiotic sensitivity, microbiology, simulation, online, undergraduate

INTRODUCTION

Teaching experimental sciences to students is firmly grounded with laboratory demonstrations of concepts and principles. In-person laboratory demonstrations allow students to learn necessary hands-on skills. However, they can be challenging for students entering a university setting with little to no experience in a science laboratory. This is especially true for microbiology, where schools may not have the necessary equipment or staff to provide basic microbiology laboratory experiences for

students before entering university. A combination of online and face-to-face interactions has been shown to enhance student learning (1). Given the complexity, depth, and importance of the microbiology curriculum across multiple disciplines, key concepts should be introduced as early as possible. To address these challenges, we made use of an in-house developed online data generator/simulator to introduce the concepts of antibiotic dose and sensitivity testing to first-year undergraduate students at the University of South Australia (UniSA). Previously these concepts were only taught to second-year students as part of a dedicated semester-long microbiology course. We used a combination of a computer simulation, which mirrored all the steps in an authentic laboratory setting, generated individualized student data, and a wet laboratory practical to teach the hands-on skills in a safe learning environment.

Due to the emergence of antibiotic resistance as a major health issue (2), this topic was chosen to test the suitability of our approach. We chose to teach two separate but related aspects of antibiotic activity. Each student was randomly allocated one of three bacteria (*E. coli*, *S. aureus*, or *P. aeruginosa*) and then tasked with determining the relationship between the concentration of antibiotic and bacterial growth. The data

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generated were then plotted and analyzed by the student, allowing teaching staff to assess the ability of the student to both correctly plot and interpret the data. This concept of antibiotic-mediated killing was then further expanded with the students determining antibiotic susceptibility testing using five commonly employed antibiotics for a bacterium according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The zone of inhibition was measured and compared to the EUCAST standard chart to determine if the bacterium was sensitive (S), intermediate (I), or resistant (R) to the individual antibiotics. With the aid of detailed written (Appendix 1 in the supplemental material) and audio instructions (Appendix 2A and 2B), both online components could be completed within 20 min. To further reinforce the concepts taught online, and to teach the important hands-on skills, the antibiotic testing was repeated in person in a microbiology laboratory over 2 days. The impact of our hybrid approach on student learning was then assessed through a written report and a questionnaire using Likert and free-text questions. Students reported a significant increase in their understanding of all processes due to the online data generator/simulation. Student feedback was very positive, and this dual approach was found to be an effective way to teach multiple concepts in a timely manner with limited background content knowledge. Given the current impact of COVID on face-to-face teaching, this approach is one way of mitigating reduced face-to-face opportunities and yet still providing students with the required skills and learning opportunities. This approach can also be applied to other laboratory-based skills in microbiology, as well as other sciences, such as chemistry, biochemistry, and molecular biology.

Intended audience

This curriculum approach is intended for first-year science students with a background in biology. However, the data generator/simulation also includes more advanced features, making our approach suitable for use with senior undergraduate students.

Learning time

We describe a hybrid online-face-to-face laboratory project that can be completed over 2 weeks. Students spend 1 h in a synchronous online lecture where the fundamental aspects of antibiotics are discussed. They then undertake the online practical simulation, which can be completed within 20 min. The face-to-face laboratory component requires an additional 2 days, the first day for teaching safety skills required for a PC2 laboratory and placement of antibiotic disks on a bacterial lawn, and the second day to measure the zone of inhibition and interpret the results.

Prerequisite student knowledge

There is no prerequisite knowledge, and students do not need experience working in a microbiology laboratory. All content regarding antibiotics and bacterial sensitivity is provided in the lecture. This covers the importance of antibiotic susceptibility testing in a diagnostic microbiology laboratory: by identifying

a causative agent and its antibiogram, they are helping clinicians to prescribe the appropriate treatment to the patient and prevent overuse of antibiotics and the emergence of antibiotic resistance. The basic principles of antibiotic susceptibility testing (agar and broth) as well as the Kirby Bauer disc diffusion method are discussed. Students are also provided with both written and video instructions to navigate the online simulation. Instructors should provide the safety information for their laboratory setting.

Learning objectives

Upon completion of this online and face-to-face practical, students will be able to:

1. Understand the relationship between antibiotic concentration and the killing of a bacterium.
2. Analyze and correctly present experimental data.
3. Evaluate and analyze and interpret the experimental data generated.
4. Understand how bacteria are identified as sensitive, intermediate, or resistant to individual antibiotics.
5. Apply their understanding to the implications of bacterial resistance to antibiotics.

PROCEDURE

The data generator was originally designed to allow students to experience the outcomes of good strategies, careful execution, and making mistakes. In creating the microbiology data generator/simulation, we took advantage of the Universal Design for Learning approach (3, 4). This framework helps developers to ensure that the needs and abilities of all learners are accommodated in a blended learning environment. In developing a teaching resource, the framework facilitates the elimination of unnecessary hurdles in learning. Our approach adopted a flexible learning environment, with information presented in multiple ways, including face-to-face, online, video explanations, and in a laboratory setting. We also provided students with several options to demonstrate their learning. Each session generates data consistent with the process the user follows and incorporates authentic, random, and systematic errors. The foundation of the data generator are rules that govern the interaction between individual components. There are no predetermined outcomes; for example, the fate of bacteria in a vessel will depend on the inoculum, the concentration of antibiotics, temperature, time, etc. The data sets produced are thus both authentic and unique between sessions. This type of simulator is especially useful for creating data sets for practice calculations and analysis. In this study, the simulator permitted students to rapidly perform multiple iterations of the experimental design and implementation process. This potentially generated a large quantity of data that was sufficient to allow users to judge the validity and robustness of their experimental results.

Our developed curriculum used a hybrid approach of an online practical component that is further reinforced with a

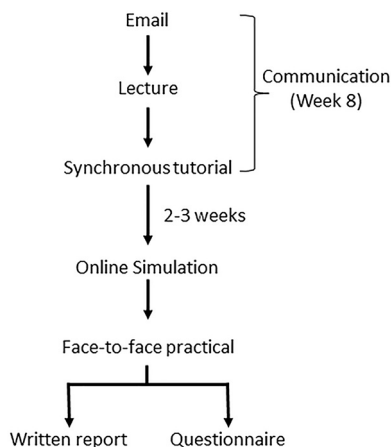


FIG 1. Flow chart outlining the timeline and steps undertaken by the students and teaching staff with the curriculum intervention.

face-to-face laboratory session, held over 2 days (Fig. 1). This practical was included in a first-year, 4.5-credit compulsory course that introduces broad content knowledge in multiple disciplines, such as biochemistry, immunology, and microbiology. Hence, an overview of the microbiology content is covered over 2 weeks. The lecture provided a brief background on antimicrobial susceptibility testing highlighting some of the theoretical and practical concepts, including the importance of identifying resistant pathogenic bacterial isolates and how this facilitates the correct choice of antibiotics to treat infections. The online data generator/simulation teaches students two aspects of fundamental microbiology: first, the relationship between antibiotic dose and inhibition of bacterial growth using a single antibiotic, and then bacterial resistance against a spectrum of clinically used antibiotics. Following the online component, students learn about bacterial growth as a lawn and how to use antibiotic disks and zones of inhibition. These results are generated over 2 days.

Materials

Each student will need the following:

1. A computer with internet access.
2. A videoconferencing platform (e.g., Zoom) for the synchronous online lecture (Can also be completed face to face).
3. Access to a word processor and graphing program (e.g., Microsoft Excel).
4. A laboratory with standard 10-cm agar plates and preprepared bacterial lawns of *E. coli*, *S. aureus*, or *P. aeruginosa*. Antibiotic disks (amikacin, ampicillin, ceftazidime, cefoxitin, gentamicin, imipenem, nitrofurantoin, piperacillin-tazobactam, tetracycline, and trimethoprim-sulfamethoxazole) and a 37°C incubator. A standard ruler or calipers can be used to measure the zone of inhibition following incubation. Appendix 3 lists the ATCC reference numbers and catalog details for all antibiotics used.

Student instructions

Students work individually during the synchronous lecture, online simulation, and hands-on laboratory component. Detailed student instructions for the online and laboratory session can be found in Appendix 1.

Faculty instructions

A flow chart outlining the steps undertaken by the instructor and student are shown in Fig. 1. Additional instructions, commentary, and advice for the successful implementation of the course are given below.

Suggestions for determining student learning

The student's performance in the laboratory practical report and written feedback was used to evaluate the effectiveness of the simulation. We developed a grading rubric to determine if students met the desired learning objectives for this assignment (Appendix 4). The rubric addressed the ability of students to correctly plot the experimental data for each antibiotic tested. This also included their ability to identify a linear relationship between dose and zone of inhibition. The students also had to demonstrate an understanding of the basic mechanism of action of the antibiotics in preventing bacterial growth. This question was then linked to their understanding of why some bacteria were able to grow in the presence of one antibiotic but not others. This was further developed through use of reference tables, which required some determination of antibiotic activity.

Designing the research project

This curriculum project would fit well into any introductory biology course. This is especially true for first-year courses, where limited time may be available to provide students with more detailed microbiology instruction. *E. coli*, *S. aureus*, and *P. aeruginosa* were chosen as they are problematic nosocomial pathogens and often exhibit multidrug resistance. They cause respiratory, urinary, and blood infections (5, 6) and hence need to be routinely diagnosed in a clinical sample. We recommend introducing the fundamentals of bacteria to students, the use of antibiotics in the treatment of bacterial infections and the issues associated with emerging antibiotic resistance in treating disease.

Implementing the curriculum project

The data generator/simulation can be accessed at the following address: https://mauriziounisa.github.io/Prof_Issues/. For both the dose-response and antibiotic resistance aspects, students were randomly allocated 1 bacterium and 1 antibiotic for the dose curve analysis and the same bacterium for the resistance testing using the Kirby-Bauer disc diffusion test (7). In the face-to-face session, the laboratory manual indicated which list of antibiotics should be used for each bacterium as well as

TABLE I
Summary of student responses to the Likert based questions (5-point scale used)

Question	Responses	Mean	SD	SEM
The introductory video helped me to use the simulation effectively.	52	4.442	0.7775	0.1078
The simulation enhanced my learning of the lecture/practical material and made the concepts clear to me.	52	4.385	0.7959	0.1104
This computer-based approach should be used to introduce other practical exercises.	52	4.135	0.8863	0.1229
I found the simulation easy to use.	52	4.115	1.003	0.1391
Before the simulation, my understanding of antibiotic testing was	52	3.346	0.8137	0.1128
After the simulation, my understanding of antibiotic testing was	46	4.37 ^a	0.6095	0.08987

^aStudent's *t* test $p < 0.0001$. The 1 score shift indicates that students self-reported moving from "good" to "very good" after using the simulation.

their optimal concentration for testing. On day 2, the diameter of the zone of inhibition for each antibiotic was measured and then compared with standard EUCAST breakpoints (8) to determine if the bacterium was S, I, or R to an antibiotic (Appendix 3).

Safety issues

The procedures and content were designed to comply with the American Society of Microbiology Guidelines for Biosafety in Teaching Laboratories. Since students will be using live bacteria, they should be familiar with standard laboratory safety protocols and wear personal protective equipment (glasses, gloves, laboratory coat) and should understand basic safety protocols within a microbiology laboratory. At the end of each day in the laboratory, the bench is wiped down with 80% (vol/vol) alcohol.

Ethical clearance

Ethical clearance for this study was granted by the University of South Australia Human Ethics Committee (application number 204123).

Potential extensions

The online data generator/simulator includes additional features that allow for it to be used for more senior students. This includes a Bunsen burner, a loop that can be used to collect colonies from a virtual bacterial plate that is then placed into a rack, and sterile tubes containing a user-specified volume of water, saline, or McFarland turbidity standard. A bacterial suspension can then be generated, and a swab then used to streak over the entire surface of the plate to prepare a bacterial lawn. Mueller-Hinton bacterial plates can also be generated. Liquids can also be dispensed using laboratory pipettes including P2, P20, P200, P1000, and P5000 volume capabilities. Finally, a report of all procedures can also be generated within the data generator.

DISCUSSION

Field testing

In 2021, 65 students were enrolled in the course. The mean age of students was 20 ± 4.4 (SD) years (range 18 to 43 years), with 53 females (82%), 11 males (17%), and 1 not disclosed. Of the students, 57 (88%) were local, while 8 (12%) were international students. The mean GPA for the students was 5.07 ± 1.26 (SD) (Maximum of 7).

Evidence of student learning

Student performance in the laboratory practical report and written feedback was used to evaluate the effectiveness of the simulation. The marking rubric used in assessing the reports is included in Appendix 5. On completion of the wet laboratory practical, students were invited to complete a questionnaire containing a Likert scale (5-point) and free-text questions. The questions related to their perceived knowledge, experience, and benefit in using the computer simulation to enhance their understanding of antibiotic sensitivity. Results were scaled 1 for *strongly disagree*; 2 for *disagree*; 3 for *neutral*; 4 for *agree*; and 5 for *strongly agree*. The two free-text questions related to the best and most challenging aspects of the simulation.

Of the 65 enrolled students, 52 (80%) responded to the questionnaire (Table 1). All but 2 students accessed the simulation more than once, with the average being 3.4 ± 2.4 accessions per student, while one student accessed the simulation 16 times. Students spent between 15 and 20 min to complete the simulation. Of the 65 students, 58 students submitted their written practical reports. Student performance in the written report was generally very good, with most students achieving the minimum of 50% to pass (Table 2). The mean score was 12.57 ± 2.06 (mean \pm SD) out of 15. Since students were allocated one of 3 possible bacteria, we assessed if student performance and/or the marking varied in any significant way. There were no significant differences in total score between any of the

TABLE 2

Summary data of student performance in the marked written laboratory report. No significant differences were noted between the individual bacterial species tested

Statistic	Combined results	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
<i>n</i>	58	18	19	21
Minimum Score	5	10.75	5	9.25
Maximum Score	15	15	15	14.5
Mean Score	12.57	13.21	11.99	12.55
SD	2.0257	1.278	2.967	1.435
SE of the Mean	0.2701	0.3013	0.6806	0.3132

allocated bacteria, suggesting that the data used within the simulator and the grading rubric used were both sound. Only 2 students failed the report, scoring 5 and 5.5 respectively. These low marks were due to the absence of responses to multiple questions. Some students did not submit since they had withdrawn from the course, while others were unwell and missed the session.

Written report feedback

On assessing the written reports, most students scored very well, and graphs were generally drawn correctly, with descriptive titles, legends with correct units, e.g., concentration ($\mu\text{g}/\text{disc}$), and distance (mm) being included. Answers to the written questions generally showed a good understanding of the relationship between concentration and activity (Table 3) and the basic mechanism of action. Results generated were effectively linked back to the theory provided in the introductory lecture. Students were also able to correctly score the results for the antibiotic sensitivity of the allocated bacterium as R, I, or S (Table 4). With the provision of a sensitivity table, most students were able to correctly identify the status of the bacterium against the tested antibiotics.

Questionnaire written feedback

The two free text questions related to the best aspects and areas for improvement of the process. A summary of the responses as well as two words clouds are shown below.

1. What were the best aspects of the simulation?

The ability to undertake the “experiment” as often and when they wanted, was common feedback (Fig. 2). In a physical laboratory

setting, students commonly only have one opportunity to undertake an experimental procedure. This may be due to time constraints or the cost of reagents. However, if an error is made in the process, there is no opportunity to repeat without the error and observe the changes in the result(s). In an online setting, this is no longer an issue and students can and do make multiple attempts to complete the process. This is where active learning can take place. Indeed, in a previous study, students repeated the simulation simply to access all the included written feedback provided to enhance their learning (9). Our data generator/simulation is purposely designed to give “variable” results, i.e., the same value will not be reported when the same procedure is repeated, and some variability is introduced into the results. Indeed, one student, who used the simulation multiple times, noted that the results varied each time (within a small degree) and incorrectly reported this as a “glitch” within the program. This was purposely designed to better replicate “real-world” data, which have inherent variability. This variability should be explained to more senior students but was not adopted for the first-year students as our intent was to introduce them to the key principles. Along a similar line, another student asked for us to “enable errors” in the software. As mentioned, variation has been introduced and we will include interactive feedback at key stages. For example, if the student uses a plate before lighting the Bunsen burner, then they will see contaminating colonies begin to grow.

The visual representation was purposely designed to mimic a laboratory setting in its design (Fig. 3). This was not lost on students, who commented that this helped them link the content in the simulation to a visual image of a laboratory setting and made the learning easier. Given the visual nature of the simulation, this is a key aspect of their learning.

TABLE 3

Sample student-generated testing varying dose of antibiotics against *S. aureus* and *E. coli*

Tetracycline (used against <i>S. aureus</i>)		Gentamycin (used against <i>S. aureus</i>)		Nitrofurantoin (used against <i>E. coli</i>)	
amt ($\mu\text{g}/\text{disc}$)	diam (mm)	amt ($\mu\text{g}/\text{disc}$)	diam (mm)	amt ($\mu\text{g}/\text{disc}$)	diam (mm)
30	25	15	25	200	25
35	28	20	32	250	28
40	32	25	40	300	32
45	35	30	45	350	36

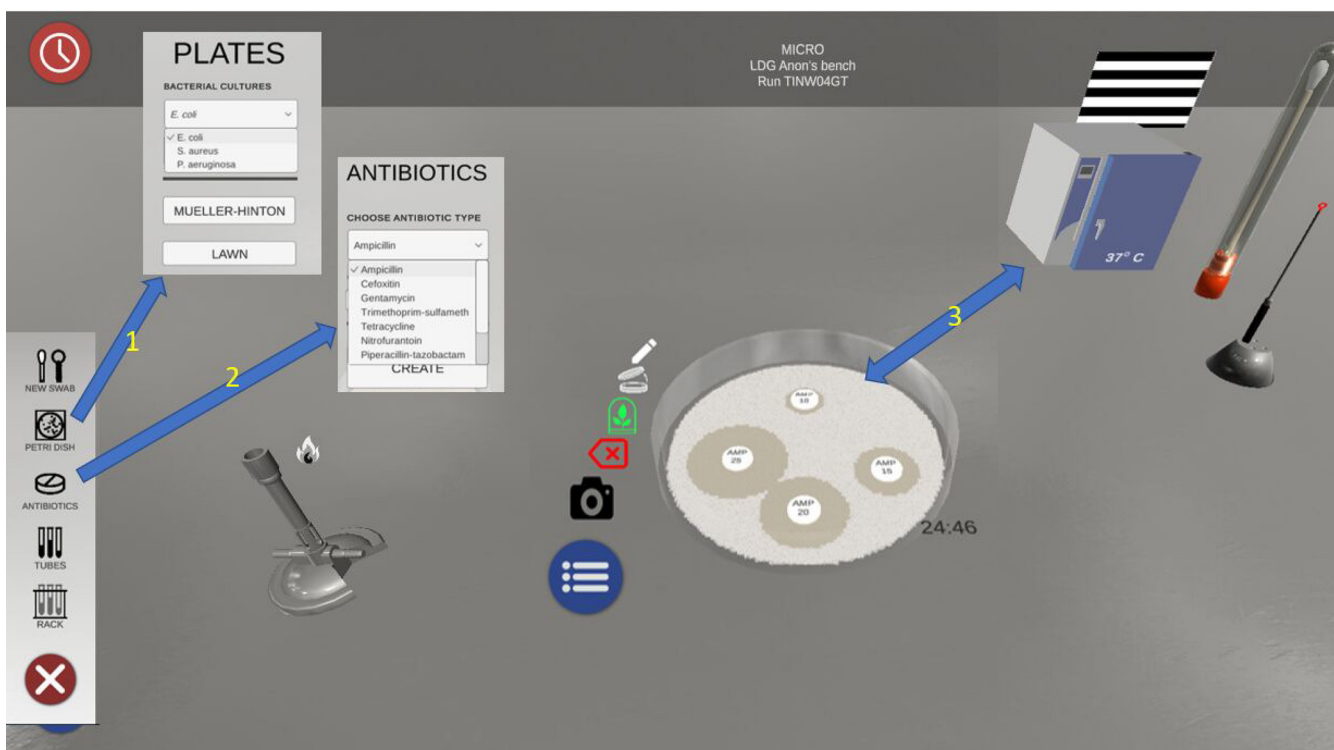


FIG 3. Screen view of the virtual microbiology laboratory. (1) On selecting the Petri dish icon, the student can choose the allocated bacterium, and then generate a lawn. (2) On selecting the Antibiotic icon, the student can select the antibiotics to be tested against the given bacterium, as well as specifying at what concentration they should be used. (3) After placing the disks, the plates are incubated and then, once removed, the zones of inhibition can be seen. Other more advanced bench features, such as the Bunsen burner, swab, and loop can also be seen; however, they were not used in this practical session.

would have used an online data generator/simulation, as well as undertaken this type of experimentation, it was key that the information presented was clear and could be followed easily. This was the rationale behind using two modes of presentation, as it can be easier to show movements (such as placing a disc onto the plate) in a video than in written instruction. In addition, the process of measuring the zone of inhibition was clearly shown, which helped clarify the process when in a real lab setting. This is evidence of authentic learning via this new method.

Linked to the instruction, the design of the simulation and its ease of use were highlighted by 11 (17%) of students. With the introduction of a new approach, ease of use is key. The aim is for learning to take place readily, rather than having students struggle with their interaction with the software and virtual environment. It was also noted that the simulation was found to be interesting, interactive, and fun by students, all of which help student cognitive engagement (10).

Due to the nature of antibiotic testing, results are not seen until 18–24 hr have elapsed. Within the simulation, time could be accelerated (up to 5,000x) so that 24 h would elapse in approximately 15 s. This allowed for results to be generated quickly and, if required, be repeated within a short time frame, again in stark contrast to a real lab setting.

2. How could the simulation be improved? Feedback was also obtained from students on how the simulation could be improved (Fig. 4). The placement of the antibiotic disks onto the virtual plate could be challenging, both in terms of the

mouse-driven deposition as well as in the generation of the final data. If placed too close to the edge of the plate, an incomplete zone of inhibition is seen (Fig. 5). This does not prevent the collection of valid data if one full axis could be measured. It should be noted, however, that this is not an issue with the data generator/simulation, as this can occur in the wet laboratory setting as well.

To measure the zones of inhibition, the students were required to take a screen capture and then print the screen, which was attached to their written report. The size of the antibiotic disks in the data generator/simulation was 10 mm, while those used in the laboratory were 6 mm in diameter. This meant some simple mathematical manipulations were required. In the future, we will make the online disks 6 mm and implement an on-screen ruler function, which will allow direct measurement of the zones of inhibition.

Additional information about the rationale for the steps was also requested by some students. This additional information will be added to both the simulation through a help function button (“?”) as well as the written laboratory instructions in future iterations.

Potential modifications

We did note one fundamental misconception made by 43 of 65 students (66%). Differences in the sensitivity between antibiotics were incorrectly ascribed to being due to the different concentrations used for differing antibiotics, rather than some

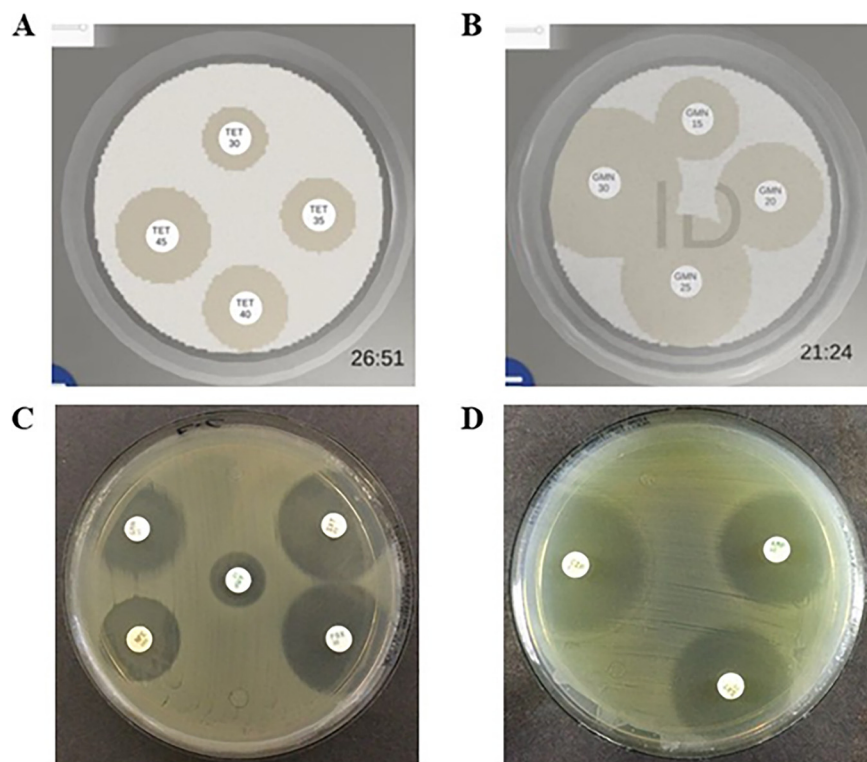


FIG 5. (A) Virtual petri dish showing well-spaced disks, with clear zones of inhibition. (B) Virtual petri dish showing zones of inhibition with overlap. These disks can still be used since one diameter can be measured. (C) Image of a petri dish containing *E.coli* treated with gentamicin (GMN; 10 μ g/disc, top left); nitrofurantoin (NFE; 10 μ g/disc, bottom left); ampicillin (AMP; 10 μ g/disc, middle); trimethoprim-sulfamethoxazole (SXT; 25 μ g/disc, top right); and ceftiofloxacin (FOX; 30 μ g/disc, bottom right). (D) Image of a petri dish containing *Pseudomonas aeruginosa* treated with ciprofloxacin (CIP) (5 μ g/disc, top left); AMP (10 μ g/disc, top left); and SXT (25 μ g/disc, bottom right).

While this approach was aimed at first-year students, the data generator has additional capacity for it to be used with more senior students. For example, it can be used to teach how bacterial suspensions are compared to McFarland standards in estimating bacterial density, McFarlane broth generation, and suspension of bacteria at an appropriate density. It also allows for the generation of a hand-streaked lawn to be generated. The inherent variability in the model allows for variation in data to be generated so that a more authentic set of data is generated.

Different versions of the data generator/simulation have already been adapted to facilitate the teaching of biochemical and molecular biology concepts including enzyme kinetics, alcohol measurements, and RT-PCR data analysis. Instructors interested in making use of these other variants should contact the authors.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.8 MB.
SUPPLEMENTAL FILE 2, AVI file, 11.2 MB.
SUPPLEMENTAL FILE 3, AVI file, 17.2 MB.

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