A CURE for Meat: Comparing Bacterial Contaminants on Different Ground Beef Sources Emphasizes Process of Science and Quantitative Reasoning

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To broaden and emphasize the educational benefits of research to more biology majors in a course setting, we developed and assessed a microbiology-focused course-based undergraduate research experience that utilizes culture-based bacterial enumeration to compare contamination present on different ground beef sources (conventional versus organic). During the final 3 weeks of the quarter, students learned and practiced common microbiology techniques like dilution math, selective and differential media-based identification, and statistical analysis to evaluate data and test hypotheses. Students were assessed primarily via a formal lab report and a lab practical focused on evaluating process of science and quantitative reasoning skills. The majority of students could write hypotheses and describe variables but were challenged when asked to describe the limitations of the experiments that were conducted as part of this research project. Most students could perform Excel-based graphing and a t test, but many could not solve the complex dilution math required for this project. The greatest barriers to skills mastery represented microbiology-focused concepts, like understanding selective media biases and the nuances of multistep viable counting procedures and outcomes.

INTRODUCTION

Engaging undergraduates in course-based undergraduate research experiences (CUREs) that are meaningful, relevant, and authentic supports national calls for action and broadens access to more students (1–6). For over 20 years, our majors-serving general microbiology course has included basic surveys of ground beef for bacterial contamination. In 2014, the PBS television program “Frontline” profiled research demonstrating links between drug-resistant bacteria on grocery store poultry and pork and human disease (7–13), inspiring the development of our course-based research project evaluating ground beef contaminants. Although a large body of research has evaluated risks associated with beef production (reviewed in reference 14), few studies have described and compared bacterial contamination on conventional (CONV) beef raised with antibiotics versus those present on organic beef raised without antibiotics (ORG or RWA) (15–19). Because procedures vary, emphasizing culture-based enumeration or drug resistance assessments and also culture-independent metagenomic approaches, conclusions are challenging to compare. Where culture-dependent studies report more total contamination on CONV beef (15), others report specific differences among some drug resistance groups (16, 17), and metagenomics-based studies report few differences in drug resistance gene profiles or population distributions (18, 19).

Understanding how the industrial use of antibacterial drugs impacts food safety represents an important problem with unknown answers and broad relevance to all consumers, an essential feature of a CURE (20–22); further examples and resources are available at CUREnet (https://serc.carleton.edu/uren/index.html). To provide a unique microbiology-focused CURE in which all biology majors could participate, we developed a research-based laboratory project at Western Oregon University (WOU) to compare MacConkey-selectable bacterial colonies on CONV beef versus those present on ORG beef raised without antibiotics. Combining two basic identification tests (MacConkey-based lactose/lac phenotypes, followed by rapid oxidase/ox testing), students identified, enumerated, and analyzed three distinct groups of selected bacteria: feces-associated coliforms (lac+/ox−) or noncoliforms (lac−/ox−), or soil-associated pseudomonads (lac−/ox+) (Fig. 1). After whole classes generated and shared data sets, each student was assigned a specific question that targeted data selection, hypothesis development, and statistical testing on one media-defined contamination subset (e.g., coliforms, total fecal bacteria). Each student wrote a formal report about their research, including identifying limitations that enabled them to critically
think about experimental approaches used and those that could be used in future iterations of this work. Students who take this course are invited to continue independent research extensions, inspiring projects that explore this question using culture-independent metagenomic comparisons and drug resistance assessments, as well as expanded food comparisons to address contamination on CONV versus ORG spinach and turkey ([23]; Tate, unpublished).

Intended audience

The activity is intended for a majors-serving general microbiology laboratory and must be conducted in a biosafety level 2 (BSL-2) lab (see Safety section). Our course is taken by all biology majors in their third or fourth year. The maximum enrollment in our course is 20, with all sections taught by one instructor (Boomer). The first version of this activity was piloted in 2015 and only emphasized learning objectives about media, hypothesis and variables, and dilution math. Specific professional development (Boomer) in 2016 advanced this project via the incorporation of statistics ([24], [25]). In spring 2018, we enhanced experimental limitation and critique expectations to align with the Inquiry and Analysis University Learning Outcomes (ULO) used for programmatic and campus assessment, emphasizing the highest, level 4/capstone rubric benchmark ([26]). In 2018–2019, we received IRB approval (WOU IRB Proposal #996) to formally assess student learning and attitudes about this research project. During this formal assessment period, this course served four cohorts representing 64 students (mostly seniors) interested in a broad array of careers, including health sciences, education, and research biology.

Prerequisite student knowledge

Students must complete yearlong introductory series in biology and chemistry, and a one-term cell biology course. Foundational courses emphasize and practice process of science (hypothesis writing and testing, experimental variables) and quantitative reasoning (algebraic conversions, Excel-based graphing, and t testing). This CURE project takes place at the end of our course, after coverage of necessary microbiology lab skills emphasizing lab safety and aseptic technique using BSL-1 and BSL-2 precautions, selective and differential media and identification testing, and viable counting and dilution math (one-step, tube-to-plate, and two-step, tube dilution-to-plate). Students were also introduced to relevant bacterial pathogens, including *Escherichia coli* (lac⁺/ox⁻), *Salmonella* (lac⁻/ox⁻), and *Pseudomonas* (lac⁻/ox⁻).

Learning time

Microbiology labs are scheduled 2 days a week (Tuesday and Thursday), with each session lasting 2 h. Data collection for this project took place during week 8 of this 10-week course; a 48-h incubation and refrigeration interval is required between these lab sessions. Group discussion, data analysis, and lab report writing occurred during week 9. The lab final was administered during week 10, along with attitudinal data collection (with surveys sealed until grades were turned in). A timeline of activities and assessment is shown in Table 1.

Learning objectives

Upon completion of this activity, students will be able to:

1. Describe selective and differential media as they impact experimental limitations.
2. Perform dilution math and solve viable counting problems.
3. Write a hypothesis and describe experimental variables.
4. Analyze project data using statistics, including graphing and t testing.
5. Critique experimental limitations using understanding of microbiology and the scientific method.

While not a formal learning objective for this lab, our whole-course lab guidelines expect students to safely handle, isolate, and culture microorganisms using aseptic technique, understanding and applying BSL-1 and BSL-2 precautions.

PROCEDURES

Ground beef laboratory exercise and assessment sequence

Over 2 years (2018–2019), general microbiology students across four cohorts (*n* = 64) compared four different packages of CONV fresh ground beef chuck (about $2.50/lb) and four different packages of USDA-certified ORG ground beef (about $6.50/lb). Both products were processed by the same Oregon meat-packing company (Interstate Meat Packers, Clackamas, OR). All packages were purchased at the same local grocery store, prior to the sell-by date.
Data collection procedures: week 8, session 1

Prior to the setup lab, students completed an online prelab about methods, media, dilution math, and statistics. The lab procedures and report assignment are provided in Appendix 1, with instructor lab preparation materials in Appendix 2. One hour prior to lab (Tuesday), the instructor diluted 5 g of each kind of ground beef (CONV or ORG) in 100 ml sterile water and rotated flasks on a platform shaker under refrigeration. Half the class plated the CONV ground beef, and half the class plated the ORG ground beef (with each table of four to six students sharing aliquots from the same primary flask). Each student prepared a 1/10 dilution tube (adding 1 ml of the beef-water suspension from the primary flask to a 9 ml sterile water blank). Each student then plated five replicates from the primary flask (spreading 0.1 ml from the flask on each plate), and five replicates from the 1/10 dilution tube (spreading 0.1 ml from the 1/10 dilution tube on each plate). All plating was carried out using MacConkey media (Difco, Sparks, MD), which generally selects for Gram-negative Proteobacteria and provides differential testing for lactose utilization (i.e., lact+ coliforms like E. coli versus lact− Providencia, Salmonella, and Pseudomonas).

Data collection procedures: week 8, session 2

Following 24 h of incubation at 37°C (Wednesday), plates were moved to a refrigerator for an additional 24 h (Thursday), which provided optimum color development for lactose testing. Based on plate count best practices, the instructor selected flasks derived for ORG and 1/10 dilution tube-derived plates for CONV, discarding replicates that were either too many to count or replicates that had fewer than 5 colonies (27). Plates were redistributed by the instructor to provide each pair of students with 5 CONV beef and 5 ORG beef plates. Student pairs evaluated lactose phenotypes, counting lact+ (coliform/fecal) and lact− (fecal or soil) colonies. After each table completed lactose assessment and counting, the instructor demonstrated positive (Pseudomonas) and negative (E. coli) oxidase controls, emphasizing that the oxidase test must be read in the first 2 min because discs (BD BBL Taxo N Discs, Becton and Dickinson and Company, Sparks, MD) will oxidize over longer periods of time, forming false positives. Student pairs then performed oxidase testing on 10 randomly selected lactose-negative colonies to estimate the distribution of oxidase-positive (soil Pseudomonas) versus oxidase negative (fecal noncoliform) colonies. A representative plate from CONV beef and an identification flow chart is shown in Fig. 1. Student pairs performed dilution math to calculate colonies per gram for all categories: fecal lactose positive (lac+), soil lactose negative, oxidase positive (lac+/ox+), and fecal lactose negative, oxidase negative (lac−/ox−). They recorded all data on provided worksheets (Appendix 1) showing raw counts, oxidase test results, and estimated calculations (colonies per gram) for each group. The instructor checked all worksheets and calculations and entered group data into a common Excel datasheet, which was provided electronically to students.

Data analysis and lab report writing: week 9

For the lab report, each student was randomly assigned one of five questions (Appendix 3) in order to guide hypothesis development and data analysis. Students met as a group with the instructor during week 9 to review lab report expectations and discuss data and questions. Each student developed hypotheses (experimental and null) addressing their assigned question and used Excel to generate a bar graph showing means with standard error bars. They calculated their t test statistic and degrees of freedom for a one-tailed t test and used these values to determine and map approximate P values using standard statistical tables of the critical values for a Student’s t distribution (28).
They wrote an experimental critique paragraph describing four limitations (Appendices 1 and 3).

**Lab final and self-assessment and attitudinal survey: week 10**

The comprehensive lab final encompassed assessment of project skills, including a media and methods-based flow chart diagram (similar to Fig. 1), experimental analysis (hypothesis writing, variable evaluation), quantitative analysis (dilution math, graphing, t test), and a limitations essay analog (Appendix 4). Students also completed an ungraded survey that asked them to self-assess how well they felt they could perform project skills, as well as attitudinal questions about food safety and public health (Appendix 5). Self-assessment data were requested in the form of a Likert scale from 1 to 5; some skill-related values were converted to percentages for graphical comparisons.

**Safety issues**

All WOU microbiology labs adhere to the ASM Teaching Laboratory Safety Guidelines (29, 30). All labs utilize only laboratory-designated equipment (pens, papers, calculators, worksheets, etc.) and exclude personal items from the lab. Because students manipulated unknown colonies from ground beef that represented potential pathogens, session 2 was run under BSL-2 conditions, which included the additional necessary use of personal protective equipment (PPE), i.e., gloves, disposable gowns, and goggles. All waste was disposed of in accordance with BSL-2 regulations before decontamination via in-room autoclaving. During the spring 2018 class, the USDA recalled nearly 15,000 pounds of beef and pork from the project meat-packing source because of pathogenic *E. coli* (31), underscoring the safety issues of colony-amplifying potential pathogens from grocery store products. In response to this event, we (Kumar and Boomer) assessed ground beef microbial populations using culture-independent metagenomic approaches, demonstrating that most Proteobacteria-like isolates were *Providencia* or *Hafnia*-like (both lac′/lox′, confirming class findings) (23). We also demonstrated that most culturable isolates were resistant to one or more of six antibacterial drugs tested (23), further stressing BSL-2 safety requirements.

**DISCUSSION**

**Field testing**

Over 2 years (2018–2019), four cohorts of general microbiology students (*n* = 64) compared the numbers of MacConkey-selectable bacterial contaminants on CONV versus ORG beef. Project protocols and attitudinal and self-assessment surveys were cleared in accordance with the WOU IRB and followed all ASM Teaching Laboratory Safety Guidelines, including BSL-2 manipulation of unknown colonies (29, 30). All cohorts observed significantly more colonies on CONV ground beef across all contamination categories (Fig. 2), with project procedures and results most similar to culture-dependent reports assessing total contamination (15). Attitudinal surveys about these results demonstrated students were more concerned about food safety and public health as a result of participating in this project and that the majority shared data with friends and family (Fig. 3). Although most students expressed greater interest in public health and a few students continued independent research, most were seniors with defined career goals and insufficient remaining time.

**Evidence of student learning**

As outlined in Table 1, this microbiology lab project incorporated and assessed two core competencies (1, 2):
the ability to use quantitative reasoning (learning objectives b, d) and to apply the process of science (learning objectives a, c, e). The latter further aligned with campus ULO Inquiry and Analysis rubrics used for programmatic assessment (26). We assessed these skills primarily via a formal lab report [overall mastery = 80 ± 0.01% (standard error)], a lab practical (overall mastery = 77 ± 0.02%), and attitudinal and self-assessment surveys.

In terms of quantitative reasoning, we assessed students in three skills: algebraic conversions used for dilution and viable counting math (learning objective b), Excel-based graphing, and using t tests to evaluate hypotheses (learning objective d). While most students (88%) could perform one-step dilution math, 54% could perform two-step math, and only 37% could perform three-step prelab problems (Fig. 4). Three-step mastery increased to 60% on the lab final, after performing dilutions and counting data analysis and group discussions (Fig. 4). Self-assessment of dilution math was 73%, suggesting students overestimate their ability to solve dilution math problems. Solving complex dilution math was the greatest barrier for students being able to understand this research experiment. Although solution-focused dilution activities are performed in general chemistry and cell biology, there are nuances to microbiology counting (e.g., dilutions are evaluated by plating live bacteria that form viable CFU) that require understanding microbiology content.

Given foundational course exposure to Excel-based graphing and t testing, assessment findings across these quantitative reasoning categories were generally higher than those for dilution and counting math (Fig. 5). Although student graphing ability was high (report at 86% and final at 92%), they slightly underestimated this skill (84%). Likewise, students could describe and perform a t test at a high level (89%, prelab and report), but they underestimated their t test understanding and skill (83%). In terms of diagramming and interpreting t test results, students slightly overestimated this ability (self-assess at 83%, versus final at 78%), often not understanding the difference between p/crit and the p/stat. Many are so focused on the 0.05 cutoff that they fail to appreciate how significant the ground beef data actually were (P < 10^{-4} to 10^{-11}). Pedagogical discussions about these results suggest variability in how faculty teach statistics (e.g., how much t/crit/p versus t/stat/p are emphasized, whether students must map/explain data using standard statistical tables).

We defined and assessed the ability to apply the process of science in terms of three skills: (i) writing hypotheses and describing experimental variables (learning objective 3), and (ii) critiquing experimental limitations (learning objective e), which requires (iii) students understand and apply methods and concepts about selective and differential media (learning objective a) (Fig. 6). Over time, student understanding of project lab media increased about 15%, from inlab (68%) and report (69%) to final (85%), with students self-assessing understanding at 81%. Although student mastery of hypothesis writing was high (report at 83% and final at 91%), they struggled more with identifying variables (report at 66% and final at 76%), often confusing dependent and independent variables. Self-assessment reporting suggests that students are slightly overestimating these skills.
and understanding, with hypothesis writing (82%) and variable identification (79%). Of assessed process of science skills, the most significant learning increase between in-lab/report and final mastery was student understanding about media (paired t test/two-tailed, \( P < 10^{-5} \)).

In terms of discussing ground beef data, few students were able to provide in-depth experimental limitations critiques (report mastery 53%), with most (75%) focused on human error (Fig. 7). When evaluating student-described limitations, we prioritized small sample size, media bias and selectivity, color-based test subjectivity, data variability, and human error (Appendix 3). That only 60% of students recognized comparing two packages of ground beef represented a small sample size was concerning to see at this stage of undergraduate learning. Winter 2018 cohort data was excluded because so few students (5 of 20) mentioned any limitations in their discussion, causing us to revise (e.g., suggesting that we add drug resistance testing) or aspects of ground beef processing that were beyond our control (e.g., manipulating treatments at the farm or meat-packing plant). Critically evaluating these experiments requires that students understand microbiology at a deep and advanced level. If students do not grasp that MacConkey media excludes many bacterial groups, then they are not going to be able to discuss the limitations of this experimental choice at the level of a microbiologist. Based on our findings, only 20% of students recognized media-based limitations when critiquing this experiment. In contrast, assessed student understanding of methods suggested 68% to 69% mastery in lab and on the report and 85% on the final. The fact that advanced limitations critiques were weak suggested students were merely memorizing media aspects of the methods, not making higher-level connections between lab and the breadth of course content emphasizing microbial diversity.

Possible modification

Many choices we made for this project were limited by the fact that we are confined by a 10-week quarter system and this is a general core course serving all biology majors; instructors who teach semesters or who offer more advanced microbiology or molecular biology electives can readily adapt and modularize this project using a number of
model CUREs about different systems (32–34). For advanced microbiology courses with longer labs, instructors could facilitate more student-initiated experimental design and media preparation training (e.g., testing for drug resistance). That said, drug-containing media preparation involves about 4 h, with many long heating and cooling wait steps. For advanced microbiology or molecular biology courses, developing culture-independent methods would make for equally interesting extensions, including emphasizing more bacterial diversity and bioinformatics (33–35). Employing only culture-independent approaches could bypass BSL-2 safety considerations. We (Boomer and Dutton) facilitate simple BSL-1-only MacConkey-based grocery surveys in our non-majors/nursing microbiology course, wherein students wax-seal plates and only assess and compare lactose phenotypes. Lastly, data from this project was adapted for use as an online lab activity for the spring 2020 term following COVID-19 mandates issued by the state of Oregon. Students were provided with original data sets generated by 2018–2019 cohort students. We administered a synchronous lab final with a practical Excel graphing and t testing component.

SUPPLEMENTAL MATERIAL

- Appendix 1: General microbiology lab materials
- Appendix 2: Instructor lab preparation
- Appendix 3: Assigned questions and grading rubric for lab report and formal writing
- Appendix 4: Lab final
- Appendix 5: Informed consent and attitudinal and self-assessment survey

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