INTRODUCTION

The World Health Organization’s 2014 report on global surveillance of antibacterial resistance (ABR) came to two broad conclusions: 1) pathogenic bacteria are showing alarmingly high rates of resistance worldwide, and 2) current monitoring systems are inadequate (50). With regard to the latter, the report highlighted a lack of standards in ABR testing methodology and reporting and a rarity of data collected from non-clinical settings. Although the importance of ABR surveillance on a global scale is widely recognized, experts agree that current testing efforts are poorly organized and funded (38).

Crowdsourcing—specifically, recruiting undergraduates as citizen scientists tasked with collecting data during laboratory exercises in biology courses—may be one possibility for obtaining large amounts of preliminary data on the frequency of ABR in the community in a cost-effective manner. Undergraduate biology students are already participating in a citizen-science program to search for novel antibacterial agents (6), and a new initiative is underway to engage students in monitoring the frequency of ABR in soil-dwelling bacteria (7).

Citizen science initiatives are common in ecology and conservation biology (41), and are becoming increasingly important in biomedical research (31, 40). In these fields, an emerging consensus suggests that monitoring studies may
be the most appropriate and effective application for citizen science (28, 31, 41). As researchers gain experience with the crowdsourcing approach, they are developing standards for effective study design, implementation, and quality control, with a focus on data validation (8, 39, 41, 46).

From an educational perspective, efforts to crowdsource data on ABR are consistent with recent calls from the National Research Council, the National Science Foundation and the President’s Council of Advisors on Science and Technology for undergraduates to pursue authentic research experiences in their coursework (3, 32, 35). In effect, the definition of best practice in undergraduate science laboratories has evolved from traditional “cookbook exercises” to inquiry labs (e.g., 10, 27) to activities that produce potentially publishable data (9, 24). These evolving practices are consistent with appeals for students to participate in the scientific enterprise by producing and managing large datasets, performing statistical analyses, and creating graphical and other representations of data (5, 33). Student-generated data are already contributing to peer-reviewed publications (13, 29).

There are at least four reasons why cataloging the frequency of ABR is a promising way for undergraduates to do authentic research. First, the need for data is great: worldwide, more people die of infectious disease than any other single cause (51), and the rapid evolution of ABR has put human populations on the threshold of a post-antibiotic era (1, 19, 22, 50). Second, student interest is strong due to the public health implications, and even stronger if the sampled cells come from their own bodies. Self-sampling is compelling from a scientific standpoint as well because, to date, most ABR surveillance studies have focused on older individuals in a hospital setting (e.g., 15, 18, 21) or children (25, 26, 42). Relatively few datasets are available from healthy young adults (50; but see 4, 10, 12, 14, 16, 48). Third, such exercises are practical. Testing commensal (non-pathogenic) cells for ABR using susceptibility test disks is safe, inexpensive, and technically accessible relative to many other options for course-based undergraduate research experiences. Fourth, collecting simple data on demographics and lifestyle makes it possible to evaluate potential risk factors associated with carrying commensal bacteria with ABR.

This paper summarizes the descriptive microbiologic data generated by undergraduate students at the University of Washington over nine years, from 2003 to 2011, and reports the associated risk factors for colonization with antibiotic drug-resistant coagulase-negative Staphylococcus. One goal of the program is to demonstrate that citizen science can play a small role in the effort to extend and improve ABR surveillance globally.

**METHODS**

Data on ABR were generated in a laboratory exercise developed for an introductory biology course for life sciences majors at the University of Washington. The course introduces students to evolution, Mendelian genetics, and ecology and currently enrolls about 2,300 students each year. Most of the students who generated the data reported here were sophomores, aged 18 to 20, with the majority reporting an interest in pursuing careers in clinical medicine or biomedical research.

**ABR laboratory exercise sequence**

The laboratory exercise on ABR spanned three consecutive weeks:

**Week 1: Isolating coagulase-negative Staphylococcus (CoNS).** Each student brushed a sterile swab across the skin at the crease on the side of the nose, then used the swab to streak a mannitol salt agar plate. The laboratory coordinator transferred the plates to a 37˚C warm room for 24 hours and then refrigerated the plates until each laboratory section met the following week.

**Week 2: Antibiotic susceptibility testing.** Students picked a single colony from the plates they inoculated in week 1, suspended the colony of cells in sterile saline, and inoculated a Mueller-Hinton plate with this suspension to create a bacterial lawn. Either the students or the lab coordinator added disks containing penicillin, oxacillin, tetracycline, and erythromycin—one in each of the four quadrants of the plate. The laboratory coordinator transferred the plates to a 37˚C warm room for 24 hours and then refrigerated the plates until each laboratory section met the following week.

**Week 3: Data collection and analysis.** Students measured the zones of inhibition around each antibiotic disk and referred to standard susceptibility tables to determine whether the cells on their plates were susceptible, intermediate, or fully resistant. Students then used software developed in the University of Washington Biology department to input data on whether the cells were susceptible, intermediate, or resistant to each antibiotic (there is no intermediate category for penicillin). The software program then asked students to answer questions about their demographic profile and lifestyle. We expanded the list of questions asked of students over time; a copy of the questions currently used is provided in Table 1a. The database contained only de-identified data without a key or other way to trace data back to individuals.

During week 3 of the laboratory exercise, groups of four students were also provided with an Excel spreadsheet containing the full dataset archived from previous terms, and challenged to think of a question that they could answer using the data. Before beginning any data analysis, each group wrote their proposed question on a whiteboard and teaching assistants (TAs) facilitated a brief discussion. The goal of the discussion was to provide feedback on each question and ask students to defend their decisions regarding the variables
they proposed to analyze and the way they expected to do the analysis. Each group determined how to manipulate the Excel file in a way that allowed them to retrieve the data required to address their question, created appropriate graphs, and performed a chi-square analysis. When their analyses were finished, each group delivered a brief oral summary of their results to the class.

The complete Student Laboratory Manual and Teaching Assistant Laboratory Manual are provided in Appendix 1 and Appendix 2 respectively. Appendix 3 contains the lifestyle and personal history questions that students answered during Week 3, and Appendix 4 lists the pre-lab questions.

Safety issues

Safety is an important issue in citizen science. In the case of ABR testing, the major concern is that students might ingest, inhale, or transmit pathogenic strains of bacteria. We took several steps to minimize this possibility:

- All work with bacterial cells was supervised by instructional staff.
- No food or drink was allowed in the lab.
- All materials that came into contact with sampled or cultured cells were promptly disposed of.
- During week 1 of the exercise, when students did the initial sampling, lab benches were wiped down with a Staphene solution after each lab section. The benches used for the plating in week 2 were also wiped regularly with a disinfectant.
- During week 2 of the exercise, the instructor—student ratio for the antibiotic susceptibility testing step was increased to 1:4. Students were explicitly instructed to select a single, pink-colored colony on the mannitol salt plate to minimize the possibility of culturing *Staphylococcus aureus*, which forms yellow colonies on these plates. To better understand the species of CoNS that students were analyzing, in the fall of 2011 we collected the swabs that 103 students had used to transfer cells to a Mueller-Hinton plate and create a bacterial lawn for antibiotic testing. A separate subculture of these cells was sent to the Harborview Medical Center Microbiology Laboratory, Seattle, WA, for species identification using a matrix-assisted laser desorption ionization (MALDI) Biotyper.

Species identification

We used mannitol salt plates to select for commensal, non-pathogenic CoNS and minimize the possibility of culturing *Staphylococcus aureus*, which forms yellow colonies on these plates. To better understand the species of CoNS that students were analyzing, in the fall of 2011 we collected the swabs that 103 students had used to transfer cells to a Mueller-Hinton plate and create a bacterial lawn for antibiotic testing. A separate subculture of these cells was sent to the Harborview Medical Center Microbiology Laboratory, Seattle, WA, for species identification using a matrix-assisted laser desorption ionization (MALDI) Biotyper.

Data validation

One of the critical steps in any citizen-science study is for experts to check the accuracy of the data being generated (41). In this case, it was important to check the validity of the student-generated ABR data based on measuring zones of inhibition. Specifically, we compared the ABR assessments made by a sample of students (*n* = 103) during a normal laboratory experience in 2011 with those of an experienced microbiologist working at the Clinical Laboratory Improvement Amendments (CLIA)-approved Harborview Medical Center Microbiology Laboratory, Seattle, WA, using the same bacterial colonies.

Statistical analysis

We performed logistic regressions with the entire dataset collected over a nine-year period (2003–2011), including all demographic and lifestyle data except for country of residence during the past five years. Because almost no males in the sample used makeup, we coded gender and makeup use in three categories: female makeup users, females who do not wear makeup, and males. To make the analysis more tractable and interpretable, we also reduced the number of categories for predictor variables in the original survey to those listed in Table 1b. For example, we re-coded responses reporting results—that is analogous to those found in scientific publications.

To assess student progress on these learning goals, we assign course points for three assessments: a prelab quiz; an “Exercise 3 report” that students write after scoring their plates and viewing the data on the frequency of resistant cells sampled by their section; and an “Exercise 4 report” that students complete after analyzing a question inspired by the large archived dataset, performing a chi-square analysis, and generating a graph. The prelab quiz and the Exercise 3 report focus on the second learning goal; the Exercise 4 report provides information on the fourth learning goal. Progress on the first learning goal is assessed by students’ ability to complete the protocol and generate usable data.
for age as 16 to 20 or over 20, instead of the four categories in the original survey.

To investigate the relationships among antibiotics while also exploring how demographic and lifestyle factors influence the probability of resistance, we included resistance to the other antibiotics as predictors, with the response variable being resistance to the antibiotic in question scored as 1 or 0. (Note that intermediate resistance in the original data was always changed to resistant, for reasons given below.) In this way, resistance to an antibiotic is treated as a known quantity in functioning as a predictor, but as a random variable in functioning as a response. We did this to explore the prevalence of multidrug resistance.

Model-fitting began with all possible predictors and two-way interactions and proceeded using a backward selection method. We performed model selection using a standard generalized linear model (GLM; logistic regression). After choosing appropriate fixed effects via stepwise entry/exit based on the Akaike Information Criterion (AIC), we fitted each model as a random-effects model (generalized linear mixed model, or GLMM), with a random intercept for year, to accommodate the potential for different years to have different baseline levels of resistance. Therefore, all results are adjusted for inter-annual differences in resistance levels. The inter-annual differences were assumed to be distributed as N(0, σ²) on the logit scale.

RESULTS

Table 2 reports data on the species identification of tested isolates, and indicates that 88.3% of the 103 isolates checked were Staphylococcus epidermidis or S. capitus. Only one of the colonies analyzed was S. aureus.

Table 3 reports the frequency of students with CoNS resistant to the antibiotics in the study. Note that although the dataset used in this analysis has entries from 9,016 students, only 8,650 individuals were able to report data on the frequency of resistance to all four antibiotics. About 4% of students were not able to measure zones of inhibition, usually because they had failed to pick and swab a sample cell successfully or because they had failed to press the antibiotic disks into the medium firmly enough to make contact. Figure 1 shows the average percentage of resistant cells in each of the nine years analyzed here, for each of the four antibiotics analyzed. Linear regression analyses indicate that there was no significant change in the frequency of resistant cells over time to any of the four antibiotics tested (data not shown).

The correlation between student-determined ABR and the results from a certified professional clinical laboratory are shown in Table 4. The "I = R mismatches" column in the table reports the number of times a student designation of intermediate resistance conformed to a determination of resistant by the professional laboratory. The column designated "adjusted % agreement" was calculated by considering all student reports of intermediate resistance as resistant. With this adjustment, there was about 90% agreement between student and reference data for three of the four antibiotics. However, the level of agreement was notably lower for penicillin (72.6%).

Table 5 summarizes the results from the stepwise regression analyses, i.e., the best-fit model for each antibiotic tested. All factors reported are fixed effects; the codes for each factor are given in Table 1b. The estimates reported in Table 5 are log-odds ratios of resistance to a particular antibiotic when the factor changes from a reference value to the value indicated in the table. For example, if students have cells resistant to erythromycin (the code for this condition is 1), the log-odds of resistance to penicillin increases by 0.38 over that of students with cells susceptible to erythromycin. Because e^0.38 = 1.46, the odds of resistance to penicillin when resistance to erythromycin has been observed is 146% of that when resistance to erythromycin is not present—a 46% increase. The rightmost column in Table 5 reports these changes in odds of resistance, as percentages, for each predictor variable with a statistically significant effect.

Note that by convention, estimates for coefficients that do not have a statistically significant impact on the log-odds of resistance are also reported if the variable in question is part of a statistically significant two-way interaction. Data for the statistically significant two-way interactions are given at the end of each section in Table 5. These two-way interaction terms indicate that there are demographic and lifestyle factors which, acting in combination, have a significant impact on the log-odds of carrying resistant cells over and beyond the effect that each factor has alone. For example, recent antibiotic use and antibacterial soap use each significantly increase the log-odds of observing penicillin-resistant cells. There is an additional increase in the probability of carrying resistant cells if students use antibacterial soap and are over 20 years old, but a decrease if these older students have not recently used an acne medication.

The largest coefficients in each model indicate that resistance to each antibiotic accounts for a highly significant amount of variation in the log-odds of resistance to the other three antibiotics. Other estimates of log-odds in Table 5 indicate that recent antibiotic use accounts for a highly significant amount of variation in resistance to penicillin, tetracycline, and erythromycin, and that recent use of acne medication accounts for a highly significant amount of variation in resistance to tetracycline and erythromycin. Other lifestyle or demographic variables are important in accounting for resistance to specific antibiotics: antibacterial soap (penicillin), makeup (oxacillin), gender (tetracycline), and age (oxacillin).

Examples of results from univariate analyses by students, using chi-square tests, are given in Appendix 5. To evaluate student mastery of the lab’s learning objectives, we analyzed scores from a recent class and found that the mean percent correct and standard error was 81 ± 0.97 for the six pre-lab questions (n = 432) and 96.0 ± 0.27 (n = 453) for the Exercise 3 and Exercise 4 lab reports. These lab report scores were significantly higher than the average.
TABLE 1.  
Data collected from students.

### a. Data entry sequence currently used in the lab exercise

Are your skin bacteria resistant to:

| Antibiotic   | Yes | No | Intermediate |
|--------------|-----|----|--------------|
| Penicillin   |     |    |              |
| Erythromycin |     |    |              |
| Tetracycline | Yes |    | Intermediate |
|             | Yes |    | No           |
|             | No  |    |             |
| Oxacillin    | Yes |    | Intermediate |
|             | Yes |    | No           |

Sex:  
- Male  
- Female

Age:  
- 16 – 20  
- 21 – 25  
- 26 – 30  
- 31+

Which of the following best describes your living situation:

- Live alone  
- Dorm or group living involving more than 5 unrelated adults  
- 5 or fewer unrelated adult housemates  
- Family with children over 6 years of age  
- Family with children 6 years of age or younger

How long has it been since you last used an antibiotic:

- Using one now  
- Within the last year  
- More than a year but within the last 5 years  
- More than 5 years ago  
- Have never used an antibiotic

Have you used acne medication:

- Using it now  
- Used within the last year  
- Yes, but not within the last year  
- Never

Do you use makeup (foundation, blush, etc.):

- Yes  
- No

Do you regularly use antibacterial soap or cleaning products:

- Yes  
- No

Do you eat meat of any kind (red meat, poultry, etc.):

- Pretty much every day  
- Several times a week  
- Once a week or less  
- Never

If you don’t eat meat, how long has it been since you last consumed meat:

- Less than one year  
- Between one and five years  
- More than five years

If you don’t eat meat, do others in your household eat meat?

- Yes  
- No

Do you work in, volunteer at or regularly visit a hospital or long-term-care facility?

- Yes  
- No

What country were you born in? (short answer)

In what country have you primarily resided for the last 5 years? (short answer)

Month data were collected

Year data were collected

### b. Data coding used for logistic regression analysis

Are your skin bacteria resistant to:

| Antibiotic   | Yes | No | Intermediate |
|--------------|-----|----|--------------|
| Penicillin   |     |    |              |
| Erythromycin |     |    |              |
| Tetracycline | Yes |    | Intermediate |
|             | Yes |    | No           |
|             | No  |    |             |
| Oxacillin    | Yes |    | Intermediate |
|             | Yes |    | No           |

"Yes" includes samples scored as intermediate resistance.

Sex:  
- Male  
- Female

Age:  
- 16 – 20  
- Over 20

Which of the following best describes your living situation:

- Live alone  
- Living with adults  
- Living with children

Last time you used an antibiotic:

- Within a year  
- Over a year ago

Last time you used acne medication:

- Within a year  
- Over a year ago

Do you use makeup (foundation, blush, etc.):

- Yes  
- No

Do you regularly use antibacterial soap or cleaning products:

- Yes  
- No

Do you eat meat of any kind (red meat, poultry, etc.):

- Often (Several times a week or more)  
- Seldom (Once a week or less)

If you don’t eat meat, how long has it been since you last consumed meat:

- Less than one year  
- Over a year

If you don’t eat meat, do others in your household eat meat?

- Yes  
- No

Do you work in, volunteer at or regularly visit a hospital or long-term-care facility?

- Yes  
- No

Where born in the United States?

- Yes  
- No

Month data were collected

Year data were collected
scores on the other eight labs in this class (paired t-test, \( t = 9.56 \), two-tailed \( p < 0.0001 \), \( df = 442 \)).

**DISCUSSION**

The data from the three assessments suggest that the exercise contributes to important learning gains in core concepts and skills (I) and thus deserves consideration for integration into introductory biology curricula. For example, the high scores observed in the antibiotic-resistance lab reports suggest that the sequence was more effective at promoting learning than the other lab exercises in the course. Because most points in the lab were assigned for the Exercise 3 and 4 reports, and because the data analysis and interpretation questions in those reports represented some of the highest-level cognitive skills assessed in the laboratory portion of the course, the alternative explanation for the result—that the questions in the Exercise 3 and 4 reports were easier than the graded questions posed in the other labs—seems unlikely. Given the goals of the movement to incorporate authentic research experiences in introductory courses, it would be interesting in the future to evaluate how important aspects of student affect—including identity as a scientist and feelings of belonging to the research community—are or are not influenced by this relatively short-term exposure to potentially publishable research.

### TABLE 2.
Species identifications from student samples (2011 sample).

| MALDI Biotyper          | # | %   |
|-------------------------|---|-----|
| *S. aureus*             | 1 | 0.97|
| *S. capitis*            | 17| 16.50|
| *S. cohnii*             | 1 | 0.97|
| *S. epidermidis*        | 74| 71.84|
| *S. haemolyticus*       | 1 | 0.97|
| *S. hominis*            | 1 | 0.97|
| *S. lugdunensis*        | 2 | 1.94|
| *S. saprophyticus*      | 2 | 1.94|
| *S. warneri*            | 4 | 3.88|
| **TOTAL**               | 103| 100 |

MALDI = matrix-assisted laser desorption ionization.

**Several aspects of this study are encouraging with respect to the hypothesis that citizen science may have a role in contributing to ABR surveillance:**

1. The laboratory exercise is safe. All of the species listed in Table 2 are common skin commensals, and some may even be beneficial to humans (II). Although CoNS can be pathogenic in certain circumstances, this is usually in the context of biofilm formation on catheters or on prosthetic devices (20). In addition, University of Washington students began this exercise in 2001, and since then an estimated 16,000 students have performed the antibiotic susceptibility testing protocol reported here. To date, we have yet to experience a safety issue.

2. The comparative data reported in Table 4 demonstrate that when student designations of “intermediate” are classified as “resistant” (as they were in our logistic regression analyses), the student and clinical laboratory data are 89 to 92% concordant for erythromycin, tetracycline, and oxacillin susceptibility. This suggests that crowdsourced data may be of sufficient quality to inform the field of ABR resistance, using the straightforward methods described here.

3. The results reported here are consistent with some existing patterns in the literature. For example, the analysis reported in Table 5 aligns with other studies demonstrating a strong association between antibiotic use and antibiotic resistance (I, 23, 34), even in commensal organisms (30, 43).

In terms of data quality, the low level of student–clinical laboratory agreement for penicillin is cause for concern, and there is clear evidence for bias in the student data on penicillin resistance: of the 23 mismatches (among 84 comparisons),...
all but one represented the same discordance—students scored the cells as resistant while the minimum inhibitory concentrations (MICs) measured in the clinical laboratory categorized the cells as susceptible. One explanation for this pattern may be that students routinely over-inoculated the plates when creating the bacterial lawn used to determine drug resistance. Staphyloccocal resistance to penicillin is mediated by beta-lactamase, and beta-lactam-mediated resistance is highly sensitive to inoculum effects (37, 44). Testing this hypothesis can be a focus of future research.

| TABLE 4. | Comparison of student-collected data and clinically licensed microbiologist–collected data (2011 sample). |
| --- | --- |
| **n** | **# Student–Professional Mismatches** | **Raw % Agreement** | **I = R Mismatches** | **Adjusted % Agreement** |
| Penicillin | 84 | 23 | 72.6 | N/A | 72.6 |
| Oxacillin | 88 | 9 | 89.8 | 7 | 92.0 |
| Erythromycin | 87 | 15 | 82.8 | 8 | 89.8 |
| Tetracycline | 89 | 13 | 85.4 | 10 | 88.8 |

I = intermediate resistance; R = resistant; N/A = not applicable.

| TABLE 5. | Output from logistic regression models (2003–2011 data). |
| --- | --- |
| **a. Penicillin resistance** |
| **Factor** | **Estimate** | **SE** | **z value** | **Pr(>|z|)** | **Δ odds (%)** |
| (Intercept) | 0.06 | 0.11 | 0.60 | 0.55 | — |
| Tetracycline Res (1) | 0.71 | 0.07 | 10.15 | <0.001 | 203 |
| Erythromycin Res (1) | 0.38 | 0.05 | 7.31 | <0.001 | 146 |
| Oxacillin Res (1) | 1.04 | 0.12 | 8.58 | <0.001 | 283 |
| Age (2) | 0.02 | 0.15 | 0.10 | 0.92 | — |
| Antibiotic Use (2) | -0.18 | 0.05 | -3.64 | <0.001 | 120 |
| Acne Medication Use (2) | 0.21 | 0.11 | 1.93 | 0.053 | — |
| Antibacterial Soap Use (1) | 0.29 | 0.10 | 2.95 | 3.2 × 10⁻³ | 134 |
| Born in US (1) | -0.12 | 0.06 | -2.06 | 0.039 | 113 |
| Tetracycline Res (1) : Oxacillin Res (1) | -0.53 | 0.17 | -3.06 | 2.2 × 10⁻³ | — |
| Age (2) : Acne Medication Use (2) | -0.41 | 0.14 | -2.84 | 4.5 × 10⁻³ | — |
| Age (2) : Antibacterial Soap Use (1) | 0.32 | 0.13 | 2.55 | 0.011 | — |
| Acne Medication Use (2) : Antibacterial Soap Use (1) | -0.24 | 0.12 | -2.07 | 0.038 | — |
| **b. Oxacillin resistance** |
| **Factor** | **Estimate** | **SE** | **z Value** | **Pr(>|z|)** | **Δ Odds (%)** |
| (Intercept) | -4.09 | 0.18 | -22.43 | <0.001 | 289 |
| Penicillin Res (1) | 1.06 | 0.12 | 8.73 | <0.001 | 297 |
| Erythromycin Res (1) | 1.09 | 0.10 | 10.63 | <0.001 | 486 |
| Tetracycline Res (1) | 1.58 | 0.12 | 8.05 | <0.001 | — |
| Gender (F1) | 0.30 | 0.15 | 2.01 | 0.045 | 135 |
| Gender (M) | 0.18 | 0.16 | 1.13 | 0.26 | — |
| Age (2) | 0.21 | 0.09 | 2.35 | 0.019 | 123 |
| Antibiotic Use (2) | 0.39 | 0.18 | 2.15 | 0.032 | 148 |
| Penicillin Res (1) : Tetracycline Res (1) | -0.55 | 0.13 | -3.09 | 2.00 × 10⁻³ | — |
| Erythromycin Res (1) : Tetracycline Res (1) | 0.25 | 0.17 | 1.44 | 0.15 | — |
| Gender (F1) : Antibiotic Use (2) | -0.48 | 0.21 | -2.23 | 0.026 | — |
| Gender (M) : Antibiotic Use (2) | -0.16 | 0.21 | -0.76 | 0.44 | — |
Whatever the cause, the result suggests that the frequencies of penicillin resistance reported in Table 3 and Figure 1 may be inflated.

In terms of contributing to ABR surveillance efforts, we suggest that student-generated data be treated as preliminary and exploratory. Clinical studies on the frequency of antibiotic resistance employ trained technicians working in CLIA-certified laboratories using standardized protocols, reagents, and organisms. While this study lacked these features, it is based on a relatively simple and large (n = 9,016) dataset that may be of interest to biologists, clinicians, and public health officials. For example, the frequency of CoNS that scored as resistant to oxacillin, erythromycin, and tetracycline reported in Table 3 was surprisingly high, given that the source population was young, healthy, and not exposed to hospitalized patients, and had relatively low personal antibiotic exposure. To put these data in better context, however, we used CLSI MIC resistance breakpoints to calculate ABR resistance from data in the EUCAST MIC database (17), which is dominated by clinical isolates. For *S. epidermidis*, we calculated 90% penicillin resistance, 79% oxacillin resistance, 10% tetracycline resistance, and 60% erythromycin resistance. For *S. capitis*, we calculated 69% penicillin resistance, 47% oxacillin resistance, and 60% erythromycin resistance. A different source (45) reports tetracycline resistance in *S. capitis* at 25% in clinical isolates from Italy. The resistance levels reported here for oxacillin and erythromycin are substantially lower; even the “likely-to-be-inflated” ABR frequency for penicillin reported here is much lower than that observed in clinical isolates. These observations suggest that resistance patterns in healthy young adults and patients may be different and are consistent with the relatively low rates reported for oxacillin-resistance
in healthy young adults in Sweden (48). Even though rates of ABR in our population seem high, they appear to be lower than rates observed in clinical isolates. The lack of a striking difference between the student population and clinical isolates in tetracycline resistance may be due to the use of tetracycline in some acne medications.

The data reported here on frequency of ABR of all CoNS should be interpreted cautiously, however, as antibiotic susceptibility varies among the different species of CoNS, and species identification was not performed for the vast majority of isolates tested. While S. epidermidis was the most common species identified in the sub-study of specimens tested in the clinical microbiology laboratory (and is among the most resistant of the different CoNS species [36]), there were nine separate Staphylococcus species identified among the 103 isolates tested, and each may exhibit different characteristic susceptibility patterns (36). It is also important to note that students tested only a single Staphylococcus colony among all the bacteria collected from their sampling location, and therefore the data in Table 3 may underestimate the percent of students who are colonized with drug-resistant CoNS.

The results from the regression analyses should also be interpreted cautiously. Because this study is exploratory in nature, we did not correct the significance levels reported in Table 5 for multiple comparisons. Thus, it is likely that some of the “significant” results indicated in the table are spurious.

As noted earlier, however, the patterns reported here align with other studies documenting a strong relationship between antibiotic use and ABR. Our results also suggest that makeup and/or antibacterial soap use may increase the probability of observing cells that are resistant to certain antibiotics under some conditions. Although antimicrobial agents such as triclosan may contribute to the evolution of antibiotic resistance (51), and even though it is common for cosmetics to contain antimicrobials to extend shelf life and conform to consumer preferences, the types and amounts of cosmetics used by the students in this study, and the frequency of their use, varies widely. Thus, interpreting the data on makeup and antibacterial soap use is difficult due to 1) uncertainty in the data on zones of inhibition, and 2) the vague nature of some of the survey questions. Even so, the results suggest that careful experimental work might be warranted on the presence of antibiotic-resistant cells as a function of makeup or antibacterial soap use.

Despite the caveats regarding the quality and completeness of the data collected, the large number of specimens screened lends validity to the test results. At a minimum, our findings suggest that antibiotic resistance among colonizing coagulase-negative Staphylococcus spp. from healthy young adults is common and that several factors increase the risk of drug resistance among these skin commensals, including recent use of antibiotics or acne medication. Finally, the observation that resistance to one antibiotic was closely linked to resistance to other antibiotics is consistent with earlier reports of multidrug resistance among CoNS isolated from hospitalized populations (15) and supports the well-known co-localization of multiple drug-resistance genes on plasmids and other transposable genetic elements that are commonly shared among bacteria (2, 47). Molecular methods could be employed to further explore the possibility of multidrug resistance in the CoNS found in this population.

In general, the patterns uncovered in the data reported here suggest that citizen science may play a role in efforts to expand ABR surveillance, while simultaneously providing an invaluable educational experience for undergraduates. Further work with students in introductory biology laboratories, focused on incorporating efficient species identification and better data quality, may be warranted.

SUPPLEMENTAL MATERIALS

Appendix 1: Student laboratory manual pages
Appendix 2: Teaching assistant laboratory manual pages
Appendix 3: Student demographic and lifestyle survey
Appendix 4: Prelab quiz questions
Appendix 5: Sample data analyses from student-generated data—univariate analyses

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