Introduction

As we have seen during the pandemic, there is an increasing need to help students and members of the public understand microbiology. Only in recent history has there been an availability and commonplace use of antibiotics, probiotics, and prebiotics (Timmis et al., 2019). Current commercial trends for health foods use these terms as marketing tools, toting probiotics in kombucha or enhanced yogurt, and prebiotics in hand soaps. As the use of these and other microbiology terms have entered the public vernacular faster than an understanding of microbiology terms have entered the public vernacular faster than an understanding of microbiology as a science, much of the public understanding of microbiology is largely influenced by “folkbiology” (Au et al., 2008). Introducing biological topics and concepts to younger students can lead to a more positive reception to the information, as was observed in a study introducing the concept of evolution (Nadelson et al., 2009). As students’ understanding of topics can also influence others’ understanding within the home, we have targeted a game-based framework for introducing the concept of antimicrobial resistance to students in middle and high school.

Using games to introduce students to the concept of antibiotic resistance has been suggested as an excellent way to engage students and raise their awareness of the topic (Molnar, 2019). This approach has also been shown to be effective in bolstering understanding of the topic in undergraduate classrooms (Govindan, 2018). Typically, however, the learning approach for microbiology concepts is largely reliant on laboratory-developed techniques for culturing microorganisms (Couto, 2017; Marvasi et al., 2017; Petersen & Chan, 2020; Williams & Stavrinides, 2020). This approach, while effective in undergraduate laboratories, has significant safety considerations and requires expensive disposables (e.g., petri dishes). This can make the culture-based approach inaccessible outside of college classrooms. When given the opportunity to explore and engage in science activities, students feel empowered and develop the communication and critical thinking skills that are needed to make meaningful connections (Trattner, 2015), making the development of safe and easily implemented learning approaches essential. The approach we describe here features hands-on learning, an essential teaching method that benefits students of all ages without the need for dedicated laboratory space. This activity targets students in grades 6–8 due to its alignment with the Next Generation Science Standards (NGSS) and the developmental stages of adolescents, but it can be amended to be appropriate to a larger range of K–12 classrooms.

The concept of antimicrobial resistance aligns well with the content of grades 6–8 life science standards in the NGSS and allows students to learn and understand organism structures, behavior, heredity, evolution, and...
Antimicrobial Resistance: The Basics

The first mass-produced antibiotic was penicillin, a chemical that is taken medicinally for the purpose of inhibiting the replication of bacterial pathogens, bacteria that are harmful to the host organism. Penicillin was first discovered for its ability to inhibit the growth of Staphylococcus spp. by the scientist Alexander Fleming in 1928 (Bennett & Chung, 2001). By the 1940s, penicillin had been commercialized (Windels et al., 2019) and was used to combat all manner of bacterial infections. Penicillin binds a subunit of the protein transpeptidase (Figure 1). Transpeptidase initiates the cross-linking of bacterial cell wall peptidoglycan. Peptidoglycan is a rigid mesh-like layer that forms around the membrane of bacteria, providing both physical protection as well as preventing intrusion into the cell by unwanted chemicals or biologicals. By blocking the cross-linking in peptidoglycan, penicillin prevents the formation of bacterial cell walls. This method of action stops bacteria from replicating since they can’t form a new cell wall for the new cell, and it can lead to the breakdown of formed cell walls. Unfortunately, this inevitably leads to selection for bacteria that are resistant to the inhibitory action of the antibiotic. More than half of the bacterial isolates of Staphylococcus pyogenes, a species of bacteria in the genus Staphylococcus, from clinical cases were already resistant to penicillin by 1948 (Barber & Rozwadowska-Dowzenko, 1948). While penicillin is an illustrative example, the story of discovery to resistance is similarly short for many of the antibiotics that are used for clinical cases today (Davies & Davies, 2010).

Antibiotic resistance within a bacterial community is not “black and white;” there are shades of gray. When we measure antibiotic resistance, we measure the minimum inhibitory concentration (MIC) of an antibiotic. This translates to how much of the chemical we need to add before it stops the replication of bacteria in the measurement environment. When exposed to tolerable concentrations, where bacteria can temporarily withstand the lethal effects of antibiotics without being able to replicate, bacteria can persist longer than the antibiotic’s dosage time (Windels et al., 2019). This

Figure 1. There are two major sub-groups of bacteria, gram-positive and gram-negative. Gram-positive bacteria have a very large cell wall that forms the outer barrier around the cell membrane, as shown in this figure. The cell wall is composed of multiple structures, but most simply, it is polysaccharides (sugars) that are held together by amino acids (the building blocks of proteins). In a normally functioning bacterial cell, the transpeptidase protein subunit, shown here in light green, links the amino acid chains between sugars (the linker is shown here as a dark green line between cell wall amino acids), holding the cell wall together. When penicillin, a chemical used as an antibiotic, is added to the system, the beta subunit (the purple square) binds to the transpeptidase subunit so that it can no longer link the amino acids. This leads to instability in the cell wall and prevents new cell walls from forming. See the online version of this article to view the figure in color.
has real-world implications when we are prescribed antibiotics. For example, if you are prescribed antibiotics for seven days and you only take them for five days, then there is a chance that the treatment will be non-lethal for the pathogen that was making you sick. Over time, this leads to higher MICs for antibiotics among pathogens, since the longer bacterial populations are exposed to an antibiotic without dying, the more likely it is that they can adapt to a higher concentration, mitigating lethal effects and maintaining this phenotype in the population. This was observed in another bacterial species, Escherichia coli, where it took only 44 hours for a population of the organisms to adapt to 3 MIC units of trimethoprim and 264 hours (about 11 days) for the population to adapt to the much higher dose of 3000 MIC units (Baym et al., 2016). This example illustrates how quickly bacteria can adapt via successive mutations to higher concentrations of antibiotics (MacLean & San Millan, 2019) and how important it is to ensure that we do not expose bacteria to antibiotics that they may survive.

Further complicating the emerging issue of antimicrobial resistance, it can spread among bacteria. Bacteria can pass pieces of DNA between themselves in a process known as horizontal gene transfer (HGT). This is similar to lateral gene transfer, or the process of inheriting genes from a parental cell, except that it is possible between unrelated bacteria. HGT occurs via three pathways: transformation, transduction, or conjugation. Transformation occurs when a bacterial cell “picks up” a loose piece of DNA (Dutta & Pan, 2002). The loose pieces may be in the environment because another cell lysed (i.e., the cell membrane fell apart when the cell died). Transduction occurs when a bacteriophage, a virus that infects bacteria, carries the DNA from one bacterial cell to the next (Dutta & Pan, 2002). Conjugation occurs through physical contact between bacterial cells, where the DNA is transferred from one cell to the other along a conjugation pilus (Dutta & Pan, 2002). Transfer of a gene that encodes for antibiotic resistance can occur via any of the three pathways of HGT. HGT can even occur between commensal (“good” bacteria) and pathogenic (“bad” bacteria) (Von Wintersdorff et al., 2016). However, HGT is statistically more likely to occur between more closely related organisms. This, like other ecological disruptions and selection pressures, can lead to changes in the overall community landscape.

Class Discussion

Before introducing the activity, the initial classroom discussion focused on first understanding what misconceptions students had about microbial life. Students were asked to think-pair-share using the topic question, “Where do you think microbes live?” Various possible environments were presented to the students (e.g., streams, soil, plants, and the human body). This segues into the topic of conversation, which is microbial life in and on the human body. Drawing on previously established knowledge of the scientific process, students developed a hypothesis answering the question, “How many cells in the human body are bacterial vs. human?” Students utilized visuals to answer this question using a human outline (Figure 2). A drawing component was incorporated into the discussion because drawing has been shown to increase engagement with scientific topics (Ainsworth et al., 2011).

Following hypothesis formation, students were introduced to literature on the topic, suggesting that the answer was 3:1 (Rosner, 2014). Students compared this result with their hypothesis and then were introduced to a more recent paper by Sender et al. (2016) that suggests the correct answer is closer to 1:1. This approach helped build students’ understanding of the self-correcting process of science, whereby the scientific literature is a conversation, a concept that students entering undergraduate studies usually misunderstand (Glaze, 2018). We encourage, prior to the implementation of this classroom activity, that further literature be searched, as this particular topic is the source of much confusion and misunderstanding within the field. See, for example, more recent classroom module papers citing higher statistics (Shoemaker et al., 2020).

Other literature, such as the work of Almeida et al. (2019) and Pasolli et al. (2019), was presented to the students to introduce another concept: the microbiota of the human body are not only numerous but also diverse.

Having been introduced to microbial diversity and abundance, we introduced students to the idea of ecosystem disturbance. In the framework of this classroom module, the ecosystem disturbance is an antibiotic. Antibiotics decrease both microbial diversity and abundance in the human body ecosystem. Finally, we introduced the concept of horizontal gene transfer as an antagonistic action that protects the microbial community from antibiotics via antibiotic resistance genes. Using this new information, students developed a hypothesis to answer the question, “What happens to the bacteria in the human body after their ecosystem is disturbed by an antibiotic?” Then, students developed a plan for data collection and hypothesis testing using the activity framework below.

○ Activity Description

Set-Up

The materials required for this active-learning approach to antibiotic resistance have a one-time cost under $100 (see Table 1) and are reusable. Materials, such as the plastic storage boxes, may be alternatively replaced with lower-cost items (e.g., wide shoe boxes) if those are available, increasing accessibility.
Prior to implementing this game in the classroom, the instructor separates beads into each plastic box, limiting the color and shape varieties to between eight and eleven; see the list of “good” and “bad” bacteria for an example (Figure 3). This creates a “diverse” but manageable microbial community. Copy, print out, and laminate the human outline (Figure 2), list of “good” and “bad” bacteria (Figure 3), and the datasheet (see Supplemental Material with the online version of this article). If the bead set purchased and divided into each box does not match, an alternate list of “good” and “bad” bacteria will need to be made for the microbial community. Each box should also receive one paper (bath) cup.

**Procedure**

Students in groups of two to four each receive a microbial community box. Cooperative gameplay is best achieved in groups of four, as two students can perform the role of the antibiotic (paper cup) and two students can perform the role of the antibiotic resistance genes. On the first turn of the game, one bacterium (bead) has the antibiotic resistance gene. This bead should be removed and set aside in a “safe” pile. This action is followed by a “dose” of antibiotics (student performs one scooping motion to remove beads with the cup); the bacteria in the cup are now dead and should be placed in the “dead” pile. Gameplay continues iteratively, where two bacteria will develop antibiotic resistance and be “safe” on the second turn, three on the third turn, etc. Keeping in mind the concept of adaptation, save all of one kind of bacteria before saving another. For example, if starting with a purple bead, then all the purple beads available in the box are placed in the “safe” pile before they pass the antibiotic resistance gene to, for example, the blue beads.

End gameplay after completing ten turns, simulating finishing a round of antibiotics. Return the “safe” bacteria to the box, now simulating what is left of the microbial community following the introduction of antibiotics. Using this collected data, graph on the y-axis how many bacteria are left alive, and on the x-axis how many turns were completed. Graph this number using one color of dry-erase marker if using laminated sheets. Next, using the provided

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**Table 1.** Materials and associated cost for a classroom size of 16 with groups of four students. Printer ink and lamination services are assumed to be provided by the school; where this is not the case, the overall price of the activity should still remain low.

| Materials Needed                             | Number Needed | Approximate Cost | Source                        |
|----------------------------------------------|---------------|------------------|-------------------------------|
| Plastic Craft Storage Box (Flat Inside Bottom) | 4             | $35.86           | https://amzn.to/2W5Tb77       |
| Assorted Beads                               | 1 bag         | $5.02            | https://amzn.to/3EHjh1M       |
| Paper 3 oz Bath Cups                         | 8             | $9.99            | https://amzn.to/39zh22h        |
| Laminated List of “Good” & “Bad” Bacteria    | 8             |                  |                               |
| Laminated Datasheets                         | 8             |                  |                               |
| Laminated Human Outline                      | 16            |                  |                               |
| Permanent or Dry-Erase Markers               | 16            | $8.48            | https://amzn.to/3AAoE0p        |
| **Total Cost:**                              |               | **$59.35**       |                               |

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**Figure 3.** Divide beads into sets of eight to eleven different colors and/or shapes per box. Create a list, as shown here, that assigns each color/shape combination to either “good” or “bad” bacteria. This creates a diverse but manageable microbial community within the ecosystem.
list, graph the number of “good” and “bad” bacteria that remained in the microbial community in two other colors.

Following the first round, depending on student level and comfort with the concepts presented, the instructor may choose to change several variables including, but not limited to, the number of “doses/turns” of antibiotic taken (length of disturbance), the efficacy of horizontal gene transfer in the community (rate of adaptation), or the efficacy of the antibiotic (severity of community disruption).

Wrap-Up
Once students have completed two or three rounds of the game, each group develops a summary of their results and whether they supported or did not support their hypothesis. Groups share their data and results with the class. Student observations have included that horizontal gene transfer of antibiotic resistance genes is “random” or is not selective for the “good” bacteria over the “bad” bacteria. Similarly, they have observed that the “antibiotic” is also not discriminatory since the cup simulates a broad-range antibiotic. Depending on student level, this can segue the conversation toward discussing the two types of bacterial cell surface structures (gram-positive and gram-negative) and how some antibiotics are meant to target one over another. Depending on the unit, discussion can also include adaptation or population ecology. Clean-up is easy; students return all beads, cups, and other supplies to the game box, and laminated sheets are wiped clean using a 70% isopropanol solution.

Participants’ Experiences
Data were collected from 50 students at a diverse middle school in eastern North Carolina. Students were presented with the above-described discussion topics and then participated in the activity led by a PhD student from East Carolina University. Of the student-respondents, 58% agreed or strongly agreed on a five-point Likert scale that the activity taught them something new about STEM. Over 50% of students reported that the activity inspired them to talk about the topic with others and look for more information about the activity. This suggests that the activity successfully raised students’ awareness of the ongoing issue of antibiotic resistance.

Conclusions
Teaching concepts such as microbiology can also include presenting students with real-world examples that they have likely already encountered in their day-to-day life. Here, we present a framework for introducing students in middle and high school to the concept of antibiotic resistance in microbial populations within the human body in a meaningful way that is both fun and inexpensive. The hands-on components in this activity are essential for this age group; doing, experiencing, and using their senses helps make relevant connections between what they are learning in the classroom and what they experience outside the classroom (Davidson, 2020). This technique also allows for students with differing abilities to participate and learn from the activity. On average, exceptional students and mainstream students prefer using hands-on learning methods and manipulatives instead of traditional learning methods in the classroom, thus improving knowledge retention and test scores (Garrity, 1998; Daviso & Textor, 2013). Further analysis of the outcomes of these active-learning methods in science support that students comprehend more scientific knowledge, acquire a love of science as a discipline, and make better connections and applications of science within other aspects of their lives (Trattner, 2015). By applying these techniques, students develop science literacy and have the potential to become stewards of the discipline as adults, as evidenced by our short-term data that they were compelled to share their new findings outside of their classroom. The issue of antibiotic resistance will continue to be relevant in the foreseeable future. Exposing students to this topic increases literacy in a current, real-world issue that students can take clear action steps toward combating.

Safety Issues
None.

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References
Ainsworth, S., Prain, V. & Tytler, R. (2011). Drawing to learn in science. Science 333, 1096–1097.
Almeida, A., Mitchell, A.L., Boland, M., Forster, S.C., Gloor, G.B., Tarkowska, A., et al. (2019). A new genomic blueprint of the human gut microbiota. Nature 568, 499–504.
Au, T.K.-f., Chan, C.K., Chan, T.-k., Cheung, M.W., Ho, J.Y. & Ip, G.W. (2008). Folkbiology meets microbiology: A study of conceptual and behavioral change. Cognitive Psychology 57, 1–19.
Barber, M. & Rozwadowska-Dowzenko, M. (1948). Infection by Penicillin-Resistant Staphylococci. Lancet 2, 641–644.
Baym, M., Lieberman, T.D., Kelsic, E.D., Chait, R., Gross, R., Yelin, I. & Kishony, R. (2016). Spatiotemporal microbial evolution on antibiotic landscapes. Science 353, 1147–1151.
Bennett, J.W. & Chung, K.-T. (2001). Alexander Fleming and the discovery of penicillin. Advances in Applied Microbiology 49, 163–184.
Couto, J.M. (2017). Biofilms for babies: introducing microbes and biofilms to preschool-aged children. Journal of Microbiology & Biology Education 18, 11.01.27.
Davidson County Schools (2020). Middle School Education: Developmental Characteristics. https://www.davidson.k12.nc.us/apps/pages/index.jsp?uREC_ID=799785&type=d&pREC_ID=1189121
Davies, J. & Davies, D. (2010). Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews 74, 417–433.
Daviso, R.L. & Textor, A. (2013). Modifications and Accommodations for Students with Disabilities in Vocational Education Programs. International Journal of Vocational Education & Training 21, 45–57.
Dulta, C. & Pan, A. (2002). Horizontal gene transfer and bacterial diversity. Journal of Biosciences 27, 27–33.
Garrity, C. (1998). Does the Use of Hands-On Learning, with Manipulatives, Improve the Test Scores of Secondary Education Geometry Students? In Master’s Program Action Research Project, St. Xavier University and IRI/Skylight, p. 64. Chicago: St. Xavier University.
Glaze, A.L. (2018). Teaching and learning science in the 21st century: Challenging critical assumptions in post-secondary science. Education Sciences (Basel) 8, 12.
Govindan, B. (2018). Bacterial Survivor: an interactive game that combats misconceptions about antibiotic resistance. *Journal of Microbiology & Biology Education* 19, 19.13.101.

MacLean, R.C. & San Millan, A. (2019). The evolution of antibiotic resistance. *Science* 365, 1082–1083.

Marvasi, M., Choudhury, M., Vala, N.B. & Teplitski, M. (2017). Fitness of antibiotic-resistant bacteria in the environment: a laboratory activity. *Journal of Microbiology & Biology Education* 18, 18.11.15.

Molnar, A. (2019). Antimicrobial resistance awareness and games. *Trends in Microbiology* 27, 1–3.

Nadelson, L., Culp, R., Bunn, S., Burkhart, R., Shetlar, R., Nixon, K. & Waldron, J. (2009). Teaching evolution concepts to early elementary school students. *Evolution: Education and Outreach* 2, 458.

Pasolli, E., Asnicar, F., Manara, S., Zolfo, M., Karcher, N., Armanini, F., et al. (2019). Extensive unexplored human microbiome diversity revealed by over 150,000 genomes from metagenomes spanning age, geography, and lifestyle. *Cell* 176, 649–662. e620.

Petersen, J. & Chan, P. (2020). A College–High School Collaboration to Support Authentic Microbiology Research. *American Biology Teacher* 82, 201–208.

Rosner, J.L. (2014). Ten times more microbial cells than body cells in humans. *Microbe* 9, 47.

Sender, R., Fuchs, S. & Milo, R. (2016). Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell* 164, 337–340.

Shoemaker, D.N., De Jesús, O.L.P., Feguer, L.K., Conway, J.M. & De Jesus, M. (2020). Inclusive Science: Inspiring 8th Graders from Underrepresented Groups to Embrace STEM with Microbiology and Immunology. *Journal of Microbiology & Biology Education* 21, 21.21.24.

Sung, H.Y. & Hwang, G.-J. (2013). A collaborative game-based learning approach to improving students’ learning performance in science courses. *Computers & Education* 63, 43–51.

Timmis, K., Cavicchioli, R., Garcia, J.L., Nogales, B., Chavarria, M., Stein, L., et al. (2019). The urgent need for microbiology literacy in society. *Environmental Microbiology* 21, 1513–1528.

Trattner, L. (2015). Making Science Come Alive. *Science Activities* 52, 53.

Von Wintersdorff, C.J., Penders, J., Van Niekerk, J.M., Mills, N.D., Majumder, S., Van Alphen, L.B., et al. (2016). Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Frontiers in Microbiology* 7, 173.

Williams, A.N. & Stavrinides, J. (2020). A Microbial Sampling and Community Reconstruction Activity for Introducing Students to the Emerging Field of Metagenomics. *Journal of Microbiology & Biology Education* 21, 21.21.37.

Windels, E.M., Michiels, J.E., Van den Bergh, B., Fauvart, M. & Michiels, J. (2019). Antibiotics: Combating tolerance to stop resistance. *mBio* 10, e02095-02019.

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