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Microbiology: Discovering antibacterial agents

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What can we do about the antimicrobial resistance crisis? What does it take to develop a new medicine? Can we fight bacteria with everyday substances or even foods? Find out with these engaging microbiology activities.

Even though microorganisms can't be seen with the naked eye, bacteria are the most abundant organisms on the planet. While many of these organisms are harmless to people, and may even be beneficial, such as our gut bacteria,^[1] some cause disease. Since the discovery of penicillin, we have been able to treat bacterial infections with antibiotics. However, antimicrobial resistance has become widespread, and we are once again starting to see life-threatening bacterial infections.^[2,3]

According to a recent report by the European Medicines Agency (EMA), infections by multidrug-resistant bacteria are estimated to cause 33 000 deaths in the EU every year, with an annual cost due to healthcare expenditures and productivity losses estimated to be approximately €1.5 billion.^[4] In fact, the World Health Organization (WHO) has declared that "[Antibiotic resistance is one of the biggest threats to global health, food security, and development today.](#)"

These activities enable students to explore this serious issue in a hands-on manner. The first activity introduces some of the basic principles and techniques, while, in the second, they get to do their own research by generating and testing hypotheses on potential antimicrobial compounds. The accompanying worksheets provide background information and guided discussions.



Safety note

In addition to following your usual laboratory and biosafety precautions:

- Do not incubate plates at human body temperature – this reduces the risk of culturing pathogenic bacteria.^[5]
- Dispose properly of leftover antibiotics and bacterial cultures to avoid releasing antibiotics or resistant bacteria into the environment.
- After incubation, tape the lids onto the petri dishes to avoid them accidentally opening. Do not tape before incubation to avoid promoting the growth of anaerobic organisms.

Activity 1: Antibiotics and resistance

The aim of this activity is to demonstrate the action of antibiotics and dose–response relationships and to explain the danger posed by antimicrobial resistance. The activity should only be carried out if the antibiotics and bacterial plates can be properly disposed of! If not, use Worksheet 1 before moving to Activity 2. The activity is suitable for students aged 14–19 and will take two lessons to complete (one to set up the incubations; one to analyze and discuss the results).

Materials

- Petri dishes with agar growth medium
- Disinfectant, such as 70% ethanol.
- Bacterial cultures (best if at least one Gram-positive and one Gram-negative per student is used)
- Plate spreader or inoculating loop
- Antibiotic tablets
- Thick paper and a paper punch
- Tweezers
- Measuring cylinder, beakers/flasks, and pipette for preparing serial dilutions
- Physiological saline solution (0,9% NaCl) or water
- [Worksheets 1](#) and [2](#) and resources on antimicrobial resistance, such as those from the US Food and Drug Administration ([FDA](#)) and the [WHO](#)^[6,7]

Procedure

Lesson 1

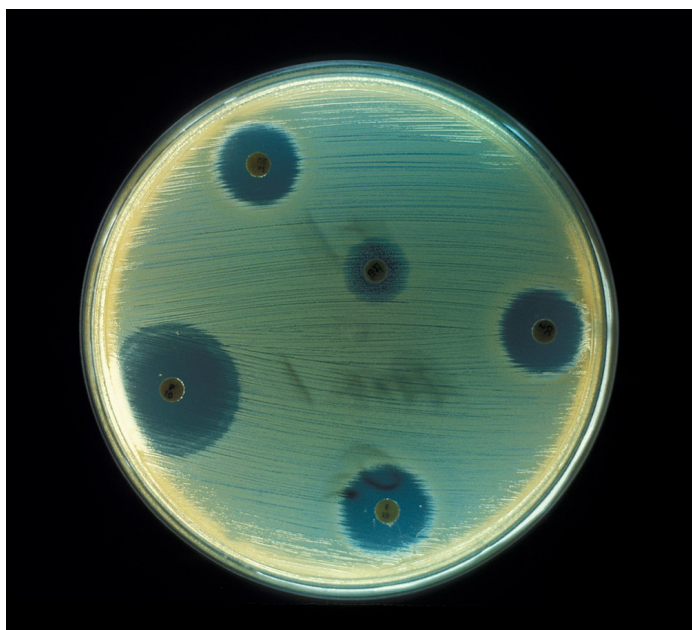
1. Sterilize your lab bench or surface by spraying it with ethanol and wiping it down with paper. Also disinfect your hands with an alcohol-based hand sanitizer for at least 20 seconds.
2. Using a sterile loop or spreader, touch the bacterial culture and spread it on the plate. It may be better for the teacher or technician to do this step beforehand. See the resource section for detailed protocols.
3. Label the bottom of the dish with the name of the group and the antibiotic chosen. Always label the bottom of the dish in case the lids get separated.
4. Cut several circles of thick paper per plate with the paper punch. Label them with a pencil (control and antibiotic at different dilutions).
5. Dissolve 300 mg of an antibiotic tablet into 200 ml of saline solution or water. Solid tablets should be crushed with a pestle and mortar; gel capsules can be gently twisted apart to pour out the powder inside.
6. Make serial 1 in 5 dilutions to get a range of samples of 1/1, 1/5, 1/25, 1/125, and 1/625.
7. Soak the thick circle paper in this solution. Use tweezers to place the disk on the plate spread with bacteria.
8. Incubate the plates at a constant temperature for 24 hours or until bacterial colonies are visible. If necessary, the supervisor can take them out and keep them in the laboratory fridge until the next lesson. Store the plates upside down to avoid condensation dripping onto the bacteria.
9. Hand out Worksheet 1 and the resources on antimicrobial resistance, which can be discussed at this point or at the end of lesson 2.



Image courtesy of the authors

Lesson 2

10. Take a picture of the plates and measure the diameter of the inhibition zone with a ruler (without opening the plate).



An antibiotic test plate showing clear circles with no bacterial growth of inoculated *Staphylococcus aureus* surrounding antibiotic-soaked paper disks

Don Stalons/CDC

11. Students can research the antibiotic and guess the group of bacteria (gram positive or negative) present in the dish. Later this can be checked with gram staining if feasible.
12. Have students fill out Worksheet 2.

Discussion

This can be done after setting up the plates or just before analyzing them. Go through the provided worksheet to analyze the results and discuss the danger posed by antimicrobial resistance. Discussion questions include the following:

- Aside from resistance, what other consequences are there of taking antibiotics when not necessary?
- In many countries, it is illegal for pharmacies to sell antibiotics without a prescription from a doctor for these reasons. Is this allowed in your country? Do you agree?
- What about antibiotic use in this activity, could that lead to antibiotic resistance? If so, what could we do to prevent this?

Activity 2: Testing substances for antibacterial activity

Having understood the need for the development of new antibiotics, students now get a chance to investigate substances for antibacterial action, followed by a discussion of the key principles of drug development and the difference between any substance that shows activity and a medicine. They will also learn to formulate hypotheses, plan experiments to test them, and analyze the results. Students find it particularly engaging to be able to come up with their own ideas of substances to test.

The activity is suitable for students aged 14–19 and will take three lessons to complete.

Materials

- Agar plates seeded with bacteria, prepared as in Activity 1
- Bacterial cultures (e.g., *Bacillus cereus*)
- Plate spreader or inoculating loop
- Substances to test for antibacterial activity
- Implements for preparing the substances (e.g., chopping board or pestle and mortar)
- Thick paper and a paper punch
- Tweezers
- Physiological saline solution (0,9% NaCl)
- Disinfectant solution (e.g., 70% ethanol)
- [Worksheet 3](#) (and [Worksheet 1](#) if skipping Activity 1)

Procedure

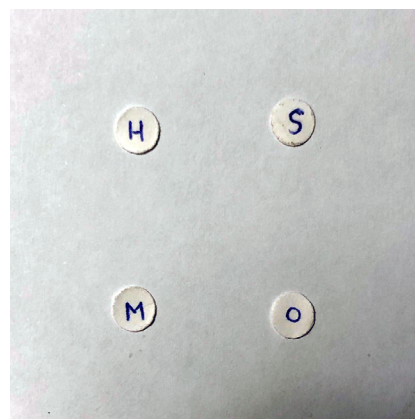
Lesson 1

1. If you haven't done Activity 1, first discuss the problem of antibiotic resistance with your class and the need for new antibiotics. Worksheet 1 can be used for this purpose.
2. Ask the students what substance in their homes they might like to test for antibacterial activity. They might already have some ideas of things they think might be antibacterial and might suggest cleaning products, cosmetics (like mouthwash), or foods.
3. Draw up a list of substances to test. Exclude very toxic or corrosive substances like rat poison or toilet cleaner. Suggestions that are nice to include are salt; mouthwash (with alcohol or alcohol-free); an antibacterial soap; a natural soap (some bacteria can feed on the soap!); and various foodstuffs like garlic juice, honey (with sugar solution to compare?), cabbage juice, a ground spice like turmeric or cloves, various essential oils, lemon juice, and apple juice.



Image courtesy of the authors

4. Have students make predictions for each substance regarding whether it will show antibacterial activity. They can do some research first or just guess based on folk wisdom or their own ideas. This is a good opportunity to discuss reliable sources and not believing everything they read on the internet, including 'published scientific papers'. Not all scientific journals have rigorous quality control! Some good references for antimicrobial activities of foodstuffs include these papers on [spices](#) and [honey](#).^[8,9]
5. Have students start to fill out table 1 of Worksheet 3. Any answers they don't know can be guessed, researched, or left blank.
6. Decide whether to use the substances pure or diluted. Maybe try one of each?
7. Students can be assigned to bring in different substances to the next lesson, or the teacher can provide them.
10. Using a marker pen, label the bottom of the dish with the name of the group and the substance chosen. After that, cut four circles of thick paper per plate and label them with a pencil.



Labelled paper circles. These are labelled with a pen but a pencil is better since it won't run in certain solvents.

Image courtesy of the authors

Lesson 2

8. Sterilize your lab bench or surface by spraying it with ethanol and wiping it down with paper. Also disinfect your hands with an alcohol-based hand sanitizer for at least 20 seconds.
9. Using a sterile loop or spreader, spread the bacteria on the plate. It may be better for the teacher or technician to do this step beforehand. See the resource section for detailed protocols.
11. Prepare the substances at the decided dosage and set them aside. See note below.
12. Soak each paper circle in the corresponding solution or saline solution as a control. Place on the surface of the agar.
13. Incubate the plates at a constant temperature for 24 hours or until bacterial colonies are visible. If necessary,

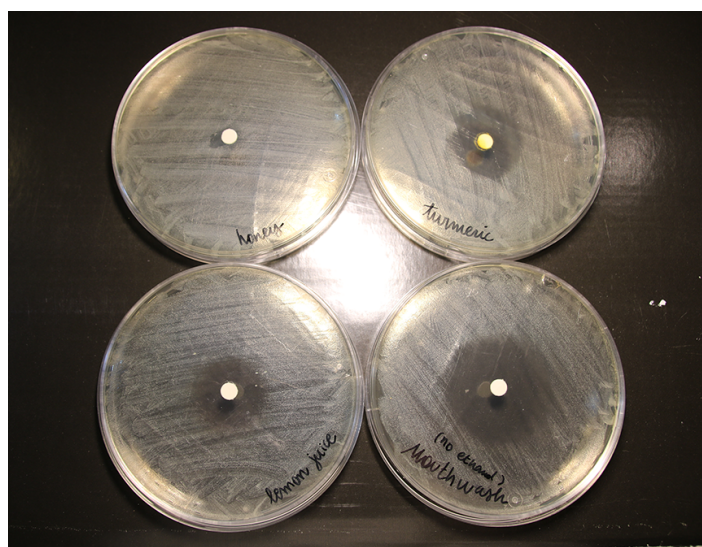
the supervisor can take them out and keep them in the laboratory fridge until the next lesson. Store the plates upside down to avoid condensation dripping onto the bacteria.

Note on preparation of the substances

- You can try to classify the substances that are going to be tested as oil and water based. The active components of solids, like spice powders, may be water or oil soluble. In this case, prepare water-soluble substances in saline solution and oil-based substances in a neutral oil (for example, sunflower oil, which will also be the control for these substances).
- Fruit/vegetables should be squeezed to extract juice. Harder things like cabbage can be grated first. Extra care should be taken if blades or graters are used.
- For citrus fruits, both the juice and oil from the peel can be tested. Strips of peel can be removed and bent (wear safety glasses to release the oils).

Lesson 3

14. Take a picture of the plates and measure the diameter of the inhibition zone.



Examples of plates tested with (clockwise from top left) honey, turmeric, lemon juice, and alcohol-free mouthwash

Image courtesy of the authors

15. Have students complete Worksheet 3 with their results.

16. Work through the discussion questions on Worksheet 3.

Discussion

The discussion on the worksheet is intended to help students understand drug development and the wide gap between a substance with a given activity and a useful medicine. The same principles apply to substances with anticancer activity. This knowledge should help them critically evaluate media reports of the amazing disease-fighting properties of various substances. <<

References

- [1] Stark LA (2010) [Beneficial microorganisms: countering microbephobia](#). *CBE Life Sciences Education* **9**: 387–389. doi: 10.1187/cbe.10-09-0119.
- [2] Chokshi A et al. (2019) [Global contributors to antibiotic resistance](#). *Journal of Global Infectious Diseases* **11**: 36–42. doi: 10.4103/jgid.jgid_110_18.
- [3] Aslam B et al. (2018) [Antibiotic resistance: a rundown of a global crisis](#). *Infection and Drug Resistance* **11**: 1645–1658. doi: 10.2147/IDR.S173867.
- [4] Information on antimicrobial resistance from the EMA: <https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/antimicrobial-resistance>
- [5] CLEAPSS safety information on how to handle microorganisms: <http://science.cleapss.org.uk/resource/SSS001-Microorganisms.pdf>
- [6] FDA resources on combating antimicrobial resistance: <https://www.fda.gov/consumers/consumer-updates/combating-antibiotic-resistance>
- [7] A WHO fact sheet on the antibiotic resistance crisis: <https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance>
- [8] Liu Q et al. (2017) [Antibacterial and Antifungal Activities of Spices](#). *International Journal of Molecular Sciences* **18**: 1283. doi: 10.3390%2Fijms18061283
- [9] Nolan VC, Harrison J, Cox JAG (2019) [Dissecting the Antimicrobial Composition of Honey](#). *Antibiotics* **8**: 251. doi:10.3390/antibiotics8040251

Resources

Experimental procedures

- Read this simple procedure for [pouring Agar plates](#).
- Use this CLEAPSS resource on [how to inoculate plates](#).
- Watch a nice video demonstrating how to [set up and perform](#) these experiments using aseptic techniques.

- Use the CLEAPSS safety information on [handling micro-organisms](#) or a more extensive [microbiology safety info](#) from the Association for Science Education.
- Read this [extensive microbiology resource](#) from ASSIST.
- Use this protocol for [Gram staining](#).

Related articles from *Science in School*

- Create a living piece of 'agar art' with microbes living on our hands: Duarte A, Madureira AM (2019) [Painting in a petri dish](#). *Science in School* **46**: 48–55.
- Look at real-life applications of school mathematics to understand how diseases spread: Kucharski A *et al.* (2017) [Disease dynamics: understanding the spread of diseases](#). *Science in School* **40**: 52–56.
- Explore the antimicrobial properties of some home-made soups: Extance A (2020) [Soup – an evidence-based medicine?](#) *Science in School* **50**.
- Read Understand articles on modern drug design:
 - Sucunza D (2014) [Inspired by nature: modern drugs](#). *Science in School* **28**: 40–45.
 - Houlton S (2019) [The changing technologies of drug design](#). *Science in School* **46**: 25–28.

Classroom resources

- Watch a video about why [antibiotic-resistant bacteria](#) are developing.
- [Watch a TED-Ed animated video on a possible solution to the antibiotics-resistance problem.](#)
- Find out more about the [antibacterial power of honey](#) with this engaging video.
- Watch an animated video by Kurzgesagt explaining [how antibiotic-resisting bacteria developed](#).
- Watch a video on the [working mechanism](#) of antibiotics.
- Watch an animation by the FDA on [antibiotic resistance](#).
- Watch an engaging [whiteboard video](#) explaining antibiotic resistance.
- [Take a quiz on antibiotic use.](#)
- [Find some information on the history of antimicrobial resistance, with dates for when resistance was first discovered for different antibiotic classes.](#)
- [Find details on antimicrobial resistance in Europe.](#)
- [Learn about different steps involved in drug development and the importance of pharmacokinetics.](#)

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