Antimicrobial Action

Activity Description & Estimated Class Time

Over the course of two 50-minute class periods, students will see the action of antimicrobial agents. Students apply paper disks soaked in antimicrobial materials to agar plates that have been inoculated with bacteria and fungi from cheese, then analyze results. Some antimicrobials kill the bacteria and fungi over an extended period (ex. antibiotic ointment) while others act more quickly (ex. alcohol, mouthwash). These results provide clues about how to use various antimicrobials effectively.

Correlations to NC Science Standards

LS.8.1.1 Construct an explanation to compare the basic characteristics of viruses, bacteria, fungi, and parasites relating to the spread, treatment, and prevention of disease.

Learning Target

Students will demonstrate knowledge and understanding that

- Antimicrobial agents are used to kill bacteria and fungi
- Different antimicrobials act in different ways. To reduce the spread of bacteria and fungi, each antimicrobial must be used appropriately.

Brief Science Background

Bacteria and fungi (but not viruses) can be grown in a relatively controlled fashion in Petri dishes filled with agar (see **Preparation** section below). Agar is a gel-like substance derived from certain kinds of red algae. A good source of non-harmful microbes is non-processed cheese, available in any grocery store. Blue cheese is especially good. Cheeses are made from milk that is cultured with friendly bacteria, usually various species of Lactococcus, Lactobacillus, and/or Streptococcus. Bacteria can reproduce very rapidly, doubling their numbers as quickly as every 20 minutes. The bacteria on an agar plate will be visible in 16-18 hours.

Other bacteria and fungi may grow on the agar plates in addition to those from the cheese. These contaminating organisms are not a problem because antimicrobials affect all bacteria in the same way. **However, warn students not to leave their plates uncovered and not to touch the agar in the plates.** When they add the paper disks, everything must be ready in advance. To prevent spores in the air from falling on the plates, raise the Petri dish covers slightly while holding them over the bottom plate, work quickly, then lower the cover.

To investigate the effects of various antimicrobial chemicals, students will apply paper disks that have been soaked in the antimicrobials to an inoculated agar plate of microbes. If a chemical kills microbes, students will see a clear area extending beyond the edge of the disk, called a zone of inhibition. Each student should use 3 disks, each with a different chemical, on their third of the agar plates. The disks should be 1-2 cm apart, so that zones of inhibition do not overlap.

This investigation does not test overall effectiveness of the antimicrobials. Some of them—antibiotic ointment, for example—contain fairly stable chemicals and are carried in a material (petroleum jelly) that does not disappear rapidly. Antibiotics are used for long-term effectiveness. Isopropyl alcohol and mouthwash evaporate quickly and are useful only immediately, on contact. They inhibit bacterial growth only briefly. Afterward, bacteria can continue growing as they dissipate. Students might conclude from this that they should wash their hands regularly, not just once every few days!

Part 1 — Preparing the Petri Dishes

Materials

Materials for preparing agar plates for multiple classes

- 7 bottles (125 ml) agar
- 50 Petri plates
- oven mitt
- cotton swabs
- permanent marker
- microwave oven or other heat source for melting the agar (provided by teacher)
- 1-2 ounces of non-processed crumbled blue cheese, **not blue cheese salad dressing** (provided by the teacher)

Materials for the whole class

- paper punches
- molded fiber trays
- coffee filters
- 1-oz plastic cups
- 1-oz plastic cup lids
- copies of antimicrobial code number sheets (SD 1)
- antimicrobial materials
    - Mouthwash
    - Antimicrobial Dial™ soap
    - Instant hand sanitizer
    - Triple antibiotic ointment
    - Softsoap™
    - Isopropyl alcohol
    - Other materials supplied by students or teacher—vinegar, bleach, household cleansers, disinfectants, etc.
- paper towels (provided by teacher)
- household bleach (provided by the teacher)

Materials for groups of 3 students

- 1 agar plate
- 1 permanent marker
- 1 forceps
- 3 Antimicrobial Action student activity sheet (SD 2)
- 1 pencil for marking disks (provided by teacher)

Preparation

allow approx 60 min.

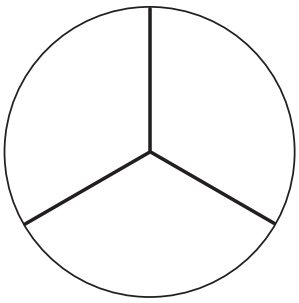
Preparing the agar plates:

1. Prepare Petri plates a day or two ahead of time. Pouring and cooling them is a job that is better not to rush.
2. Lay out Petri plates on a layer of newsprint so that they are easy to pour quickly.
3. If a microwave is not available, place the jar of agar with a loose cap in boiling water. To melt the agar in a microwave, follow the directions below **VERY CAREFULLY** to prevent the agar from exploding and coating the inside of the oven.
    - Use the oven mitt provided when handling the hot bottles
    - **LOOSEN** the cap on the bottle.
    - Heat on high for **30 SECONDS.** The agar should begin to melt.
    - Continue heating for short periods of **NO MORE THAN 15 SECONDS**. After each heating, hold the bottle with the oven mitt and swirl it gently with opening pointing away from you. Swirl **CAREFULLY**. Agar can bubble up

Preparation cont.

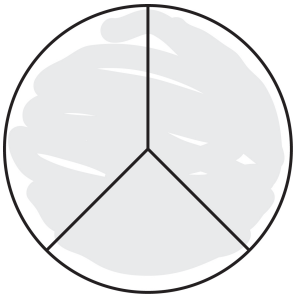
if it gets too hot. As you swirl, look for cloudiness or lingering thickness in the agar. Toward the end, heat for 10 seconds at a time.

4. Uncover the plates and pour agar into each smaller inner plate. When you pour, cover about ¾ of the bottom surface and swirl gently to spread it evenly. The agar layer should be thin but completely cover the bottom of the plate. If the outside of the agar bottle is wet, quickly dry it with a paper towel to avoid dripping outside liquid into the plates. You will especially need to do this if you heated the agar with boiling water. Immediately after pouring, replace the larger, outer cover. If lumps appear while you are pouring, reheat the agar before continuing. One bottle should make 8-9 plates.
5. After the agar cools and solidifies (about 5 minutes), check for solidity and turn the plates upside down to prevent condensation from 'raining' down on the agar. Store the plates in their plastic sleeves to keep them from drying out. Store the plates in a refrigerator if you need to keep them more than a day before use. If the agar surface is very wet when you pull the plates out to use them, shake them out onto a paper towel to get rid of the water. Avoid touching anything to the agar surface.
6. For each agar plate, use the permanent marker to mark the smaller bottom plate, dividing it into thirds.



Preparing the bacterial cultures:

1. Soak 1-2 ounces of crumbled blue cheese in a little water overnight to soften it.
2. Make a slurry of cheesy water by stirring to break up any clumps.
3. Inoculate the entire plate with the cheese slurry following the steps below:
    - Dip the swab in the slurry and then immediately and gently wipe it evenly across the entire surface of the agar. Take care not to press the swab into the agar, because that will dig into the agar. Avoid touching the agar with anything but the swab. It is not necessary to see material on the plate. The antimicrobials show clearer effects when you very lightly coat the plates. Remember that antimicrobials do not cause bacteria to disappear; they just prevent them from living and growing.



Preparation cont.

Preparing 8 work stations for each class:
(these materials will be used for all classes)

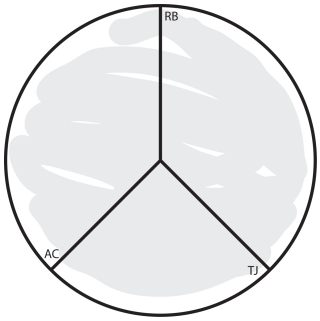
1. Label 1-oz cups with number codes to correspond to each antimicrobial material (see chart). Fill the cups ½ to ¾ full with each of the 6 antimicrobial materials provided and then cap them for storage.

Code #	Antimicrobial Material
1	Mouthwash
2	Antimicrobial Dial™ soap
3	Instant hand sanitzer
4	Triple antibiotic ointment
5	Softsoap™
6	Isopropyl alcohol

2. If students want to try other antimicrobials (vinegar, bleach, household cleansers, disinfectants, etc.), provide cups for these as well.
3. Using the molded fiber trays, set up 8 work stations. Each work station should have the following:
   - 1 paper punch
   - 1-2 coffee filters
   - Antimicrobial materials
   - antimicrobial code number sheet (SD 1)
   - paper towels (provided by teacher)

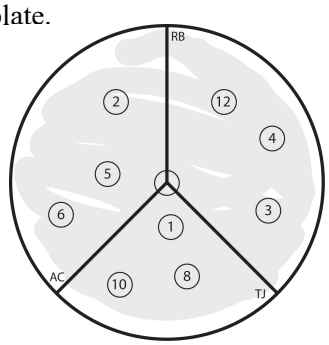
Procedure

4. Explain that the class will investigate the antimicrobial properties of different antimicrobials. Tell students that each group of three will grow bacteria on a Petri dish to test the effectiveness of antimicrobials.
5. Arrange students in groups of three. Have two or three groups at each work station. Give each group their materials.
6. Project SD 1 and have students predict which antimicrobials will be most effective in killing the bacteria. Have them rank the antimicrobials on their antimicrobial student activity sheet (SD 2) from most effective to least effective. Ask students to share their predictions with the whole class and give reasons for their predictions.
7. Ask teams to use the paper punch to punch out disks from the coffee filters. Each group needs 10 paper disks.
8. Ask students to use a *pencil* to write on the paper disks the code number of the material they will use on that disk, **one number and one material per disk**.
9. Ask students to use a permanent marker and mark a section of the Petri dish with his or her initials. **Be sure to mark the smaller bottom plate.** If you mark the larger lid, it can rotate freely, and you will not know whose samples are where!



Procedure cont.

10. **Demonstrate to students** how to use the antimicrobials:
    a. Dip the forceps in isopropyl alcohol and allow to air dry.
    b. Hold a numbered disk with the dry forceps and dip the disk into the antimicrobial material for which it is labeled.
    c. Blot the disk (or wipe it off gently) on a clean paper towel to remove excess.
    d. Place the disk in a section in an agar plate. Antimicrobial materials such as the ointment are sticky, making transfer difficult.
    e. Before repeating the process with another antimicrobial, wipe the forceps clean and dip them in isopropyl alcohol again.
11. Each team will dip nine disks, three for each student. Each student will number and dip disks in three different antimicrobials, then apply them to their section of the agar plate. Ask students to evenly distribute the disks in their section, and place them so that they do not touch. In the figure below, disks with numbers above 6 represent antimicrobials supplied by students, which have their own numbers.
12. Ask each group of three students to place a blank, non-dipped "control" disk in the center of their agar plate.



13. Collect all the plates and store them in a covered cardboard box. Put the box where it can stay warm; a window sill often provides enough warmth. You might even cover the box with a towel overnight to help keep it warm.
14. Check the cultures each day for 3-5 days. When most petri dishes have substantial growth, proceed to Part 2.

Part 2 — Analyzing the Petri Dishes

Procedure

1. Give each team their Petri dish. Allow students 10-15 minutes to observe their own plate and encourage them to look at other dishes to compare.
2. Ask students to sketch on SD 2 what they observe on their own section of the plate. Ask them to include observations about similarities and differences in the bacteria around the various disks. Students should also record questions that arise while making observations. Students may be able to measure the distance between the edge of the filter paper and the beginning of the bacterial growth (zone of inhibition).
   The size of this zone indicates the effectiveness of the antimicrobial.
3. DISPOSAL: When the students are finished, soak the plates in a bucket of dilute bleach (9 parts water, 1 part bleach) for an hour to sterilize them. Double bag the sterilized plates and dispose in regular trash.

Content Connection

1. Ask each team to rank their antimicrobials by effectiveness on SD 2. Ask teams to provide all evidence they gathered to support their rankings and explain how that evidence is relevant.
2. Have students compare their actual ranking of effectiveness to their predicted ranking. Lead a discussion in which students share what interested them most.
3. Ask students the following questions about characteristics of bacteria and fungi:
    - What basic characteristics of the cheese bacteria and fungi allowed them to spread from the cheese to the agar plate?
      All bacteria are able to reproduce. When they are in the right environment, this can happen quite rapidly, especially if they are reproducing asexually which causes exponential growth. What started as a few bacteria that are not observable can quickly become a large colony that could spread to the agar plate.
    - How might these characteristics allow us to prevent their spread or stop them from growing?
      If the bacteria are reproducing asexually then they are "copying" the same genetic material. So, if one bacteria is susceptible to the antibiotic, then they all would be. Also, if some bacteria are being stopped by the antibiotic, then that reduces the number of bacteria able to reproduce.
    - How do these characteristics relate to the treatment and spread of disease?
      When treating a disease, you want to both stop the spread (reproduction) AND destroy the bacteria that you have.
    - What might happen if a bacterium that caused a disease could not be treated or prevented by any antimicrobial? Use this as an opportunity to discuss epidemics and pandemics.
      If a bacterium cannot be killed via antimicrobial then it can spread as rapidly as it can acquire the materials it needs for survival. This would make most humans susceptible and able to spread the disease to others. Only those that happened to survive or not acquire the infection would not be hosts to the bacteria.
4. If the following ideas do not come up, also discuss with them:
    - How might you increase the effectiveness of an antimicrobial?
      To increase efficacy you would want to use the antimicrobial until all the bacteria are killed. This means using enough for long enough to kill all types, even the most resistant that linger long enough.
    - What might make an antimicrobial less effective for preventing the spread of disease?
      Effectiveness depends on using the antimicrobial long enough to kill ALL of the bacteria. Inconsistent use allows the "strongest" of the bacteria to survive and reproduce which makes it more dangerous.
    - What was the purpose of the blank disk in the center of the agar plate?What does it tell us?
      The blank portion represents a control area where no antimicrobial is placed. This can be used as a comparison point to observe the unrestricted growth of the bacteria.

SD 1

Antimicrobial Code Numbers

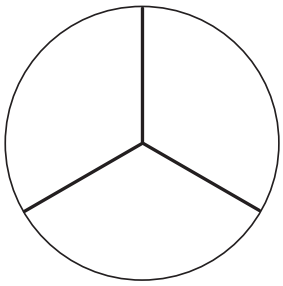
1. Mouthwash
2. Antimicrobial Dial™ soap
3. Instant hand sanitizer
4. Triple antibiotic ointment
5. Softsoap™
6. Isopropyl alcohol
7.
8.
9.
10.

SD 2 Antimicrobial Action Student Activity Sheet

Name:

1. Rank the antimicrobials from most effective to least effective. Give reasons for your predictions.
   Most Effective **Least Effective**

2. Sketch what you observe on your own section of the plate. Document similarities and differences in the bacteria around the different disks.
   Similarities **Differences**



3. What questions do you have about your observations?

4. Measure the distance between the edge of the filter paper and the beginning of the bacterial growth. This is the zone of inhibition. The size of this zone indicates the effectiveness of the antimicrobial.

5. Rank your antimicrobials by effectiveness. Provide evidence from this activity to support you rankings and explain how that evidence is relevant.
   Most Effective **Least Effective**

6. Compare your actual ranking of effectiveness to your prediction.