Investigating the Effects of Antiseptics and Antibiotics on Bacteria

Fran Maher

Introduction

The study of bacteria as agents of infectious disease is encountered in VCE Unit 3 Biology, Area of Study 2. Students should understand the role of bacteria in disease and the types of agents able to block the growth of bacteria and the spread of infection. The practical activity described here supports the achievement of this outcome and can be used as a SAC for Unit 3 Outcome 2, “A summary report of a practical activity related to bacterial response to chemical stimuli”. It can also be used for studying microbes in Year 9-10 Science.

Antiseptics and antibiotics are agents capable of preventing or slowing the growth of bacteria. Antiseptics are substances such as chlorine and phenolic compounds that are usually manufactured synthetically and applied to surfaces such as skin, floors and benches. Antibiotics are biochemicals, derived from living organisms, which act at low concentrations to kill bacteria. The first antibiotic discovered was penicillin, produced by the mould Penicillium.

Bacteria can be resistant to antibiotics due to genes that enable bacteria to destroy an antibiotic or pump the antibiotic out of the cell. These genes are usually carried on plasmids (small circular pieces of DNA carried by bacteria) that are transferred to other bacteria during the process known as conjugation. Thus bacteria can spread their antibiotic resistance to other bacteria.

Introducing students to the use of agar plates for the culture of bacteria and the concept of antibiotic resistance genes is also relevant to Unit 4. The role of plasmids as vectors for gene transfer, the role of antibiotic resistance genes as selection agents in genetic modification, and the development and spread of antibiotic resistance in bacteria as an example of natural selection and evolution can all be related back to this practical activity conducted in Unit 3.

Agar is a jelly-like substance, a carbohydrate extracted from seaweed, commonly used as a semi-solid support medium for the growth of bacteria and fungi. Agar plates contain agar and a range of nutrients to promote bacterial growth. ‘Nutrient agar’ is a common, basic agar plate designed for the growth of bacteria without special growth requirements, such as Staphylococcus and Escherichia species. When bacteria are grown as a ‘lawn’ on agar plates, they give the appearance of an opaque layer. When an antibacterial substance is present in the agar, the result is seen as a clear space where the agent has diffused through the agar and bacteria have been killed. Substances applied to the surface of an agar plate via an absorbent paper disc can diffuse radially away from the disc. When bacterial growth is prevented, the area around the disc appears clear and is called the ‘Zone of Inhibition’. This method of testing the sensitivity or resistance of bacteria to an antibiotic or antiseptic is used in medical microbiology testing of bacterial infections to identify the appropriate antibiotic to apply to an infection and in environmental microbiology to monitor the spread of antibiotic resistance in bacterial populations.

Aims

1. To grow a bacterial lawn and test the antibacterial activity of several agents
2. To observe the differences in antibiotic sensitivity of different bacterial species
3. To demonstrate different methods of spreading a bacterial lawn

Materials

Before ordering materials, decide whether you wish to use one bacterial species and compare different
agents on one species or use two bacterial species and compare the different effects of antibiotics on each species. Most antiseptics will give the same result on different species suitable for growth in the classroom. Different antibiotics may give differing results on different species.

If choosing one species, Staphylococcus albus is a good choice as it gives a reliable lawn for clear interpretation of the results. S. albus is also known as Staphylococcus epidermidis and is a member of the normal flora of human skin, nose and mouth.

Suppliers: Agar plates (already poured), bacterial cultures, antibiotic rings and blank sterile discs are available from Southern Biological. They have ratings that indicate the biosafety level for each bacterial species available.

The following procedures are written for testing two different species (halve the plate numbers if only one species is being tested) and for testing antiseptics and antibiotics on the ‘Mastring’ antibiotic rings with 6 different antibiotics.

Methods
Part A - Investigating antiseptics


2. As a group, decide on 3 agents to be tested for antibiotic activity on 2 different bacterial species. Use sterile water as the 4th agent for testing.

3. Collect 2 agar plates and the 4 agents your group will test.

4. Label the bottom of each agar plate (the part with the agar):
   a. Around the edge label with the date, group name, bacterial species.
   b. Draw diameter lines at right angles to divide the plate into four sections.
   c. Label the sections 1–4 or with the name of the agent to be tested.
   d. Record which agent is being tested in each quadrant.

5. Spread a “lawn” of each bacterial species on each agar plate using one of these methods:
   a. With a sterile swab:
      i. Dip the sterile swab into the bacterial broth
      ii. Spread the bacteria evenly over the entire surface of the agar plate with the swab
   b. With a glass spreader:
      i. Use a sterile pipette to place 2–3 drops of bacterial broth onto the agar plate

   • Liquid soaps; Bleach; Mouth wash eg. Listerine; Floor cleaners; Toilet cleaners; Ammonia; Clorox; Handy Andy; QAC (Quaternary ammonium compound) hospital grade disinfectant, Betadine topical antiseptic (available from pharmacies)
   • Use sterile water (autoclaved or boiled) as a control.
Investigating the Effects of Antiseptics and Antibiotics on Bacteria (continued)

ii. Dip a glass spreader into 70% ethanol, pass it through a Bunsen burner flame then remove from flame and let the ethanol burn off to sterilise the glass. Do not leave the glass in the flame as it may melt or will heat too much and kill the bacteria while spreading.

iii. Spread the bacteria over the surface of the agar plate – rotate the plate to allow an even spread.

6. Sterilise forceps by dipping into ethanol and burning off the ethanol (this step should be eliminated if the risk of accidents and burns is considered too high).

SAFETY when surface sterilising instruments with ethanol:

a. Use only Pyrex beakers or Petri dishes for ethanol containers for dipping objects (if ethanol is in a plastic container and catches fire, the container will also burn)

b. The tips of the forceps should face down at all times so that ethanol does not run down the instrument towards the fingers.

c. DO NOT replace the flaming object into the ethanol container.

d. If a beaker of ethanol catches fire, move students away from the area, cover the beaker with a watch glass or glass Petri dish lid, or simply let it burn off (the beaker will get very hot so do not handle until cooled).

7. Use the forceps to pick up a sterile absorbent disc, dip it into the agent to be tested, let excess agent drain off the disc and place the disc in the centre of the correct region on the agar plate.

8. Repeat for each agent to be tested.

9. Use adhesive tape to secure the lid to the agar plate or seal the edge of the plates with Parafilm (it is important that the lids cannot easily be removed during incubation and when students are viewing the experimental results after bacterial growth).

10. Place plates in the incubator (or other warm place) with the bottom (agar side) up and the lid down (this allows any condensation to fall down onto the lid and not onto the bacterial lawn). Incubate for 24–48 hours in a 37°C incubator. If the lawn has not grown into an opaque layer by then, something is wrong with the bacteria or plates.

Control plates: use one agar plate to spread a lawn of each bacterial species being investigated. Add nothing to the plate and culture with other plates to ensure that the bacteria will grow into a lawn with no manipulations.

Part B - Investigating antibiotic sensitivity: Demonstration Plates

1. Label the bottom of 2 agar plates with the name, date, bacterial species.

2. Spread a lawn of each bacterial species on the agar plates (as described above).

3. Place an antibiotic ring (with 6 different antibiotics) in the centre of each plate.

4. Seal the edge of the plate with Parafilm or adhesive tape.

5. Place plates in the incubator, bottom up/lid down.

Results

1. Draw each plate, showing the zone of inhibition (the clear area around the disc where the bacteria didn’t grow) OR take a photo of your agar plates with a digital camera (light reflection off the plastic can be a problem for getting a clear image).

2. Measure the zone of inhibition (the clear region lacking bacterial growth; measure from the edge of the disc to the growth region) for each agent.

3. Record the results in a Class Results Table.

4. Record the zone of inhibition for each antibiotic tested on the demonstration plates.

Examples of results:

Part A

Part B
Safety and organisation issues to support implementation in the classroom

Laboratory arrangement
Dispense Bacteria at one station: to avoid bacterial spills, the teacher or lab technician can dispense the bacteria onto agar plates at one station, students spread the bacteria immediately, then return to lab bench to place discs on plates. The disposal container for swabs is located at this bench for immediate disposal.

- **Advantage**: bacterial cultures, swabs and spreaders are confined to one bench.

Place individual antiseptic agents at stations around the room with one pair of forceps to stay with each reagent to avoid mixing of substances.

- **Advantage**: only one set of antiseptic agents is required.
- **Disadvantage**: movement around the room – potential for dropped plates, accidental contamination of skin and objects with bacteria.

OR

Have a set of antiseptic agents at each bench.

- **Advantage**: after spreading lawn, students return to one bench.
- **Disadvantage**: Forceps need to be washed between each agent used; Need a full set of antiseptic agents at each bench.

Bacterial growth rates
Most bacteria divide about every 20 min. Bacteria plated as a lawn should show up as an opaque layer on the surface of the agar after 18–24 hours incubation at 35–37°C.

Storage of plates before and after culture
Nutrient Agar plates: store in sealed plastic bags at 4°C. Bring to room temperature before class use. Do not let plates dry out.

After incubation: after the bacterial lawn has grown (after 24 hours incubation at 37°C), the plates can be stored at 4°C in plastic bags (agar up/lid down) until your next class. Do NOT let plates dry out.

Comparing Zones of Inhibition for antiseptics and antibiotics
Different antibiotics and antiseptics diffuse through the agar at different rates due, for example, to different molecular mass. Therefore when comparing different agents, the size of the zone of inhibition may not demonstrate relative antibacterial potency, just whether or not they inhibit growth of the bacterium.

If the agents have spread too far from the disc, and they are all effective, almost all bacteria on the plate will be killed, i.e. the whole plate may be clear, so it looks like the bacteria didn’t grow at all. This is why control plates with a bacterial lawn (but no other treatment) is required. The extensive spreading of antibacterial agents can be limited by allowing excess liquid to drip off the discs before placing on the agar.

Incubators
Not all schools have an incubator that can be set at 37°C. Any warm space will allow the bacteria to grow e.g. above a water heating unit, near a heater, in an enclosed insulated box or esky with an incandescent light. If using a sunny window-sill, cover the plates as UV may damage the bacteria and limit growth. Ensure that the temperature does not exceed 37°C.

Glass spreaders
These can be made from glass stirring rods or Pasteur pipettes by melting the glass about 5cm from the end of the glass rod, with a Bunsen burner flame, until it drops at 90°C.

<table>
<thead>
<tr>
<th>Swabs versus Glass spreaders</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>
| Swab                        | • purchase sterile  
• no ethanol flaming required  
• easier                        | • Purchase costs  
• appropriate disposal required  
• not reusable            |
| Glass spreader              | • reusable  
• spreader is sterile after ethanol flaming  
• easy to make  
• even spread  
• less biohazard waste for disposal | • ethanol flaming required – potential for burning ethanol  
• potential for ethanol spills and then burning ethanol  
• harder technique for students - requires closer supervision |