Investigation of the effect of antibiotics on bacterial growth

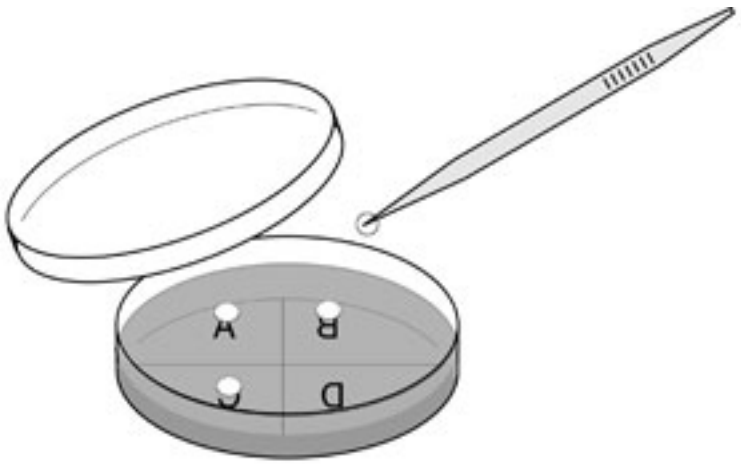
Introduction

Antimicrobials are agents that are able to kill bacteria or halt their growth. They are widely used in medicine to treat bacterial infections. In this experiment you will test different anti-microbial agents to assess how they affect bacterial growth.

Apparatus

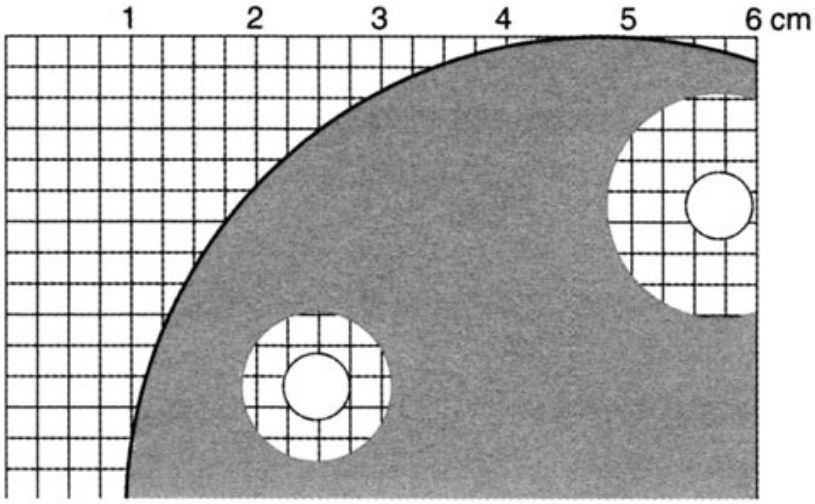
Bunsen burner
1 × pre-prepared agar plate seeded with bacteria
4 × antimicrobial agents, labelled A, B, C and D
4-8 × paper discs (Whatman antibiotic assay paper discs/ or new filter/ chromatography paper cut with a hole punch then sterilised by autoclaving)
sterile forceps
adhesive tape
marker pen
disinfectant solution and cloth

Diagram of Apparatus



Method

1. Wash your hands with the soap or handwash. Wipe down the working area thoroughly with the disinfectant.
2. Work very close to a lit Bunsen burner. Flame the forceps and use them to pick up a filter paper disc and dip the disc into antibiotic A.
3. Allow them to dry for 5 minutes on an open, sterile Petri dish, next to a lit Bunsen burner.
4. Repeat step 3 for antibiotics B, C and D.
5. Use the agar plate that has already been prepared and seeded with bacteria.
6. Turn the dish upside down. Divide the base into four sections by drawing a cross with the marker pen. Label the sections A, B, C, D.
7. Flame the forceps and then use them to pick up antibiotic disc A. Raise the lid of the Petri dish at an angle and place the disc onto the agar in the centre of section A.
8. Repeat step 5 for the other 3 discs. Make sure the discs are placed in the centre of each section.
9. Label the agar plate with your name and date. Tape the lid securely. Incubate inverted for 2-3 days at 20-25 °C.
10. Observe the plates without opening them.
11. Record the width of the clear zone around each antimicrobial. A piece of squared paper under the agar plate might be helpful here.



Analysis

1. Which antimicrobial agent was the most efficient in your investigation? Give reasons for your answer.

Risk Assessment

Hazard	Risk	Control measure
Bacteria can be pathogenic	Touching bacteria when plate is open	Wash hands Incubate plates at room temperature Seal plate so that they are not opened
Bunsen burner flame and Forceps can burn	Burning skin when placing discs on plate	Care must be taken to keep hands a safe distance away from the flame. Do not touch tip of forceps after flaming

Teacher / Technician notes

Detailed instructions are given on the link below.
http://www.nuffieldfoundation.org/practical-biology/investigating-anti-microbial-action

Making agar and pouring plates

a Calculate the quantity required and prepare just enough agar for the investigation – around 15 cm^3 for normal depth in a 90 mm Petri dish. Any surplus will keep for 6-12 months in tightly-sealed screw-top bottles if sterile.

b Weigh out the agar medium powder containing the gel and chosen nutrients, add water and sterilise the mixture for the time, and at the temperature, specified by the manufacturer.

c Heat agar and water at 95 °C to dissolve the agar. Always use a water bath to boil agar, and never add agar to boiling water.

d Stopper flasks with a well-fitting plug of non-absorbent cotton wool. Cover with greaseproof paper or aluminium foil before sterilising by autoclaving.

e After autoclaving, transfer to a water bath to equilibrate at 50 °C. Stack plates after pouring to minimise condensation except in the top plate(s).

f Warm the Petri dishes before pouring to minimise condensation.

g Keep poured plates in a sealed plastic bag until needed to reduce dehydration of the media.

Making a spread plate

1 Sterile spreaders are used to distribute inoculum of *Bacillus subtilis* over the surface of prepared agar plates. You can sterilise a wrapped glass spreader in a hot air oven or sterilise by flaming with alcohol.

2 To flame a spreader with alcohol:

a Dip the lower end of the spreader into a small volume of alcohol (70% IDA) contained in a vessel with a lid (either a screw cap or aluminium foil) or in a glass (not plastic) Petri dish with a lid. Keep the alcohol container covered and 1 metre away from the Bunsen burner flame.

b Pass quickly through a Bunsen burner flame to ignite the alcohol. Ensure the spreader is pointing downwards when and after igniting the alcohol to avoid burning yourself.

c Remove the spreader from the flame and allow the alcohol to burn off. The burning alcohol will sterilise the glass.

d Do not put the spreader down on the bench.

3 Cotton wool swabs can be used instead of glass spreaders. They may be preferable as they avoid the need for using alcohol as a sterilising agent. Prepare them by rolling small pieces of absorbent cotton wool around one end of a cocktail stick. Wrap individually in aluminium foil or place inside a universal bottle to sterilise in an autoclave or pressure cooker. These sterile swabs can then be dipped into the solution or culture to be transferred, rubbed on the surface of the agar plate, and immediately disposed of into disinfectant. (Note: Cotton buds from a pharmacist are not sterile and may be impregnated with an antimicrobial agent.)

4 Use agar plates with a well-dried surface so that the inoculum dries quickly. Dry the surface of agar plates by incubating for several hours (perhaps overnight) or put them in a hot air oven (at 55-60 °C) for 30-60 minutes with the two halves separated and the inner surfaces directed downwards.

The antibiotics can be bought as ready made discs or solutions can be made from everyday ingredients. Many types of toothpaste contain low concentrations of anti-microbials, and mouthwashes claim plaque-killing potential.

The ten spices with the most potent antibacterial effects are garlic, onion, allspice, oregano, thyme, cinnamon, tarragon, cumin, cloves and lemon grass. Many spices with relatively weak antibacterial effects become much more potent when combined; examples are in chili powder (typically a mixture of red pepper, onion, paprika, garlic, cumin and oregano) and five-spice powder (pepper, cinnamon, anise, fennel and cloves). Lemon and lime juice, while weak inhibitors themselves, also have synergistic effects.

It is also possible to investigate different dilutions of a particular anti-microbial.

Students should be made aware of aseptic techniques before starting the practical activity. It is possible that students can prepare their own pour plates and inoculate them if you wish.

Practical techniques covered

B1 Use of appropriate apparatus to make and record a range of measurements accurately, including length, area, mass, time, temperature, volume of liquids and gases, and pH.

B3 Use of appropriate apparatus and techniques for the observation and measurement of biological changes and or processes.

B4 Safe and ethical use of living organisms (plants or animals) to measure physiological functions and responses to the environment.

B5 Measurement of rates of reaction by a variety of methods including production of gas, uptake of water and colour change of indicator.