WHICH ANTIBIOTIC IS MOST EFFECTIVE?

Purpose

- To investigate the effect of different antibiotics on bacteria.

Introduction

When a bacterial infection is diagnosed antibiotics may be prescribed. Different antibiotics are not equally effective against all bacteria, so the correct antibiotic must be selected for a particular bacterial infection. In some cases the most effective antibiotic is known, but in other cases tests need to be carried out by a pathology department. In this activity you will be testing the effectiveness of several types of antibiotics on bacteria.

SAFETY

Some general points about conditions in the lab when preparing for microbiology practical work are listed below:

- *Wear eye protection.*
- *No eating, drinking, licking labels, nor chewing gum, pencils or pens.*
- *Cover any cuts or broken skin with a waterproof plaster before starting the practical work.*
- *Clear and clean the work surface that will be your workstation with a suitable disinfectant.*
- *Light a Bunsen burner at your workstation so there is an updraft away from the bench surface.*
- *Wash your hands with warm water and soap before and after the practical work.*
- *Collect all the equipment you will need, making sure glassware, Petri dishes, pipettes, agar, etc. are sterile.*
- *A jar/beaker of disinfectant should be available at each workstation for disposal of contaminated items.*

See CLEAPSS Student Safety Sheet 1 and the microbiology section in the CLEAPSS biology handbook for further details.

Do NOT open the Petri dishes once they have been incubated.

Scientific questions and information research

- *State what you are going to investigate* – you should express this as a question to answer, a problem to investigate or a hypothesis to test.
- *Research relevant information* – to help you decide how you will carry out the practical work, you might need to research the background science and methods people have used to investigate similar problems. The activity completed in Topic 4 investigating antibacterial properties of plants will provide a good start. When you write up your plan remember to give full details of any information sources you use and comment on their reliability.

Planning and experimental design

The standard method of doing this is to put discs of blotting paper soaked in the various antibiotics onto an agar plate that has been inoculated with the bacteria. Alternatively, a mast ring (a ring of paper with several 'arms', each treated with a different antibiotic) can be used.

- Design an experiment that you can use to complete your investigation – the Developing Practical Skills Support on SNAB Online will help you plan your investigation.

You will be provided with the following equipment:

- Agar plate seeded with known bacteria
- Sterile Pasteur pipette
- Bunsen burner

- Beaker of disinfectant (1% solution of Virkon™ or equivalent)
- Bactericidal soap
- Paper towels
- Marker pen
- Forceps
- Mast ring or antibiotic impregnated paper discs
- Adhesive tape
- Incubator set at 30 °C.

Make sure your plan includes a risk assessment that identifies potential safety issues and how they can be minimised.

Have your plan checked by your teacher/lecturer before you start the experiment.

Analysis and interpretation of data

- Collect data from other members of the class. Note which bacterial cultures and which antibiotics were used in each case.
- Calculate means and/or median values for each antibiotic and bacterium culture.
- Present your results in the most appropriate way including any calculated values.
- Summarise any trends or patterns in the class results.

See Maths and Stats Support Sheet 7 – averages, and Maths and Stats Support Sheet 1 – presenting data – tables in the support section of SNAB Online. Remember to record a suitable number of significant figures in measured and calculated values. If you have repeated measurements use these to comment on the significance of your results. See Maths and Stats Support Sheet 9 for an introduction to statistical tests.

Conclusion and evaluation

In the write-up of your experiment, make sure you:

- state a clear conclusion to your work that explains your data: remember to use evidence from the data and your own biological knowledge
- comment on the accuracy, precision and validity of your results
- comment on the validity of your conclusion
- comment on whether or not the outcome of your work was as you expected: if it was not, try to explain why not
- compare your conclusions with information available from other sources so that you can see if your conclusions agree or disagree with those of other scientists
- discuss any safety precautions you took during the experiment
- describe any modifications you would make to improve the quality of your results.

Questions

Q1 What factors determine the diameter of the inhibition zones?

Q2 Why were you told to incubate the plates at 30 °C, when human body temperature is 37 °C?

Q3 If you were working in a hospital laboratory and you had just carried out this test on bacteria isolated from sick patients, would you always choose the antibiotic that gave the biggest inhibition zone? Are there any other factors you would need to consider?

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Ensure eye protection is worn throughout.

Review students' risk assessments and discuss any safety considerations.

The microorganisms are a potential biological hazard. Use aseptic techniques when transferring the bacteria to the Petri dishes. Ensure students conduct practical work on trays to capture any spills. Any spills should be cleared up quickly by using 1% Virkon™ solution. At the end of the practical, re-apply the solution and leave in place for 10 minutes. Do NOT open the Petri dishes once they have been incubated.

Ensure students wash their hands thoroughly at the end of the practical.

Notes on the procedure

The aim of the practical is to show students a method of determining bacterial sensitivity to different antibiotics and to give them practice at using aseptic techniques. This could be done with antibiotic discs or the same technique could be used with antiseptics. The Student Sheet is presented as a planning exercise. Students have used similar methods in Activity 4.22 so should be able to prepare a reasonable plan for this investigation. A procedure is provided at the end of these teachers notes. Students could work in small teams to investigate different bacterial cultures and different antibiotics, then pool their results for analysis. They will need to present their requirements for equipment, including bacterial cultures, well in advance, if they are not guided on what will be available.

Although students consider allergic responses in Q3, where antibiotics are already impregnated into discs, the risk of an allergic response is not great. This is because the discs are not handled directly and no dust is created by the discs which could become airborne.

The behaviour of students must be considered. If you suspect they may attempt to lift one of the pieces of tape on a 'sealed' dish, then the dishes should be sealed around the circumference *after* incubation, before being returned for observation. Also, to prevent agar plates being removed from the lab, count all plates back in again before students are allowed to leave.

Answers

Q1 The rate of diffusion of the antibiotic will be influenced by the size of molecule, its concentration and the potency of the antibiotic. If the antibiotic is effective at lower concentrations the circle will be larger (all other things being equal). Gram-positive and Gram-negative bacteria respond differently to antibiotics.

Q2 The bacteria used in this experiment are not pathogenic strains; they grow well at 30 °C. Pathogenic bacteria grow best at body temperature. Therefore, the Petri dishes are incubated at 30 °C to avoid culturing any pathogenic bacteria that have inadvertently been allowed to contaminate them.

Q3 You may have to consider whether or not the patient is allergic to any of the antibiotics. For example, allergy to penicillin is not uncommon. You may consider the state of the patient's immune system. In patients with a weakened immune system you would not want to use a bacteriostatic antibiotic, that is, one that stops bacterial reproduction but does not kill the bacteria. Some antibiotics can be used together and produce a larger effect when combined than if administered separately. This is known as synergism.

Procedure

1. Wash your hands thoroughly. Cover the working area thoroughly with the disinfectant solution and wait for the disinfectant to act (10 minutes with Virkon™, longer with other disinfectants).
2. Prepare an agar plate seeded with bacteria. This may have already been done for you. If not, follow the instructions on the Student Sheet 'Pouring agar plates' which can be found with Activity 4.22. Label the Petri dish on the base at the edge with your name, the date and the type of bacterium it is inoculated with.
3. If not already autoclaved, sterilise the forceps by flaming them and allow to cool. Use them to pick up an antibiotic disc or mast ring. Raise the lid of the Petri dish and place the mast ring firmly in the centre of the agar; if individual discs are used they will need to be spaced evenly around the dish.
4. Tape the dish securely with two pieces of adhesive tape (but do not seal it completely), then incubate it upside down for 48 hours at 30 °C.
5. Wash your hands thoroughly and disinfect the bench again with the Virkon™ solution (leave in place for 10 minutes).
6. After incubation, look carefully at the plate, but do not open it. Where bacteria have grown, the plate will look opaque, but where the antibiotics have inhibited growth, clear areas called inhibition zones will be seen. Measure the diameter of the inhibition zones in millimetres, work out the radius and calculate the area. Present your results in an appropriate way.

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Wear eye protection.

The bacterial culture could present a biohazard and should be handled and disposed of in accordance with current guidelines on microbiology in schools.

Disposal of agar plates and other used equipment must be in accordance with current best practice.

Autoclaving is the preferred method of disposal over any chemical disinfection method.

This experiment could be done with antiseptics rather than antibiotics if not available. Paper discs produced with a hole punch are dipped in a range of antiseptics.

Requirements per student or group of students	Notes
Agar plates seeded with bacteria or equipment for their preparation (i.e. access to one of two broth cultures of known bacteria; McCartney bottle of nutrient agar; sterile Petri dish; sterile pipette)	Suitable bacteria are listed in various catalogues. Phillip Harris use *E. coli* and *Staphylococcus albus* in their kit. The Student Sheet with instructions for preparation of seeded agar plates, 'Pouring agar plates', can be found in Activity 4.22.
Bunsen burner	
Beaker of disinfectant for discarded pipettes	1% Virkon™ or equivalent. Virkon™ is widely available from specialist suppliers, including pet suppliers. Microsol® is a useful alternative.
Bench disinfectant	1% Virkon™ or equivalent. Leave in place for 10 minutes.
Soap or handwash	Hand-washing has to be reasonably thorough.
Paper towels	
Marker pen for marking Petri dishes	
Forceps (metal)	These are best provided already autoclaved within a cotton-wool stoppered boiling tube.
A mast ring or separate antibiotic discs	Separate antibiotic discs are usually cheaper, but it is harder to obtain a range of antibiotics using these.
Adhesive tape	
Incubator set at 30 °C	This will be needed for at least 48 hours.
Eye protection	