MODULE:

Chemistry and medicine: Microorganisms in/on everyday objects and a diffusion antibiogram

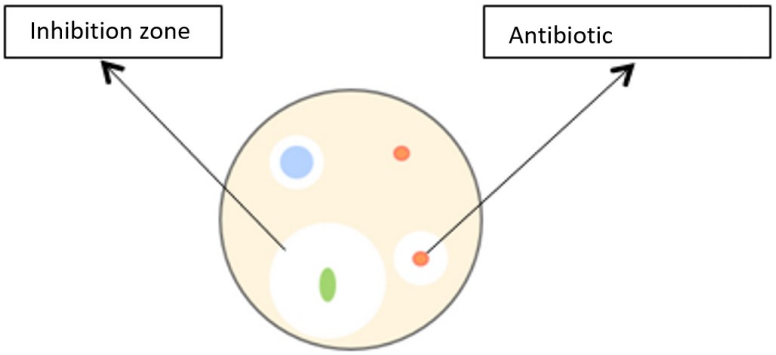
DESCRIPTION OF PRACTICAL:

A microbial culture includes microorganisms that grow in or on a culture medium. A microbial culture may be mixed or pure. A pure culture contains only one type of microorganism and the characteristics (e.g. antibiotic production, inducing diseases) of that culture are also the characteristics of that type of microorganism. The characteristics of a mixed culture, however, cannot be attributed to only one type of bacteria. Microorganisms in a mixed culture may also affect one another, thus facilitating or hindering each other's growth. The offspring of a single bacteria or cell that grew and reproduced on a solid culture medium is called a colony, i.e. a mass of cells visible to the naked eye. Microbiology examines the activity of mixed cultures, such as those usually found in nature, while a pure culture is prepared when an individual type is to be examined.

The cultivation of microorganisms provides the basis for studying them. The growth of microorganisms implies the growth of cells and an increased number of cells. It is necessary to provide the nutrients, energy source and suitable physico-chemical conditions for growth, and everything mentioned must imitate the natural environment to maximum possible degree. Every microorganism has optimal, minimum and maximum growth temperatures. Culture media are solutions of nutrients for the development of microorganisms under laboratory conditions, while the structure of a medium and its pH is adjusted to the needs of the microorganism being developed. Every medium may be prepared as a liquid, solid or semi-solid medium with respect to the amount of agar added to the culture medium. Agar causes the medium to solidify. For inoculation, i.e. the addition of microorganisms in/on a culture medium, an inoculation loop is used, also in combination with a swab or pipette.

Diffusion antibiogram is a simple way to establish bacterial resistance to antibiotics. Antibiotics are applied to an infected agar plate. An area emerges around the place of application where the bacteria did not grow; it is called the zone of inhibition. The diameter of the zone of inhibition is measured with a ruler. The agent with the largest zone of inhibition is the most effective in the destruction of that type of bacteria.

Schematic diagram of diffusion antibiogram:



MATERIAL:

- bottles with plastic caps
- test tubes
- agar
- sterile swabs
- sterile saline solution
- disinfectant soap
- disinfectant
- 70% ethanol
- garlic
- ginger
- honey
- toothpaste
- random objects to test for the presence of microorganisms

METHODS OF WORK:

Preparation of agar plates:
First sterilise the petri dishes by placing them in a microwave oven for 10 minutes. This will destroy all microorganisms. Prepare agar and pour it in liquid form in bottles with plastic caps. Then place the bottles containing agar in a pressure cooker (which should contain some 3cm of water) and heat it to obtain maximum pressure in some 15 minutes. Sterilisation under these conditions should be carried out for another 15 minutes. It is recommended that something is put under the glasses, so that they do not directly touch the bottom of the cooker. Be careful not to fully tighten the bottle caps, as they may cause the bottles to crack due to excessive pressure. If you do not intend to use the prepared agar immediately, screw down the caps after sterilisation and store the agar. It may be necessary to heat it before next use, so that it liquefies. After sterilisation, wait for the agar to cool down to a temperature allowing the bottle to be held in a hand, but do not wait too long, as the agar will solidify and you will not be able to pour it in petri dishes. Put agar in petri dishes without the previous sterilisation in pressure cooker; just mix the agar with water, heat it up until it dissolves, and pour it in a petri dish. These plates will be used in an exercise to test the efficiency of antibiotics.

When you open a bottle and transfer agar in sterile petri dishes, stand by the fire in order to ensure aseptic work. Furthermore, also work by the fire when opening petri dishes later on, unless testing for microorganisms in the air.

Effectiveness of hand washing:

1. Divide petri dishes with agar in half and mark them. One half will serve for fingerprints before washing and one for after washing. Be careful to imprint fingers before washing always on one half for comparison.

2. Imprint fingertips on the first petri dish and then wash your hands under running water for 1 minute, after which again imprint the fingertips on the same hand, but washed, on the plate.
3. Repeat the experiment on the second petri dish in the same way using a disinfectant soap, whereby washing your hands with disinfectant soap under running water for 2 to 3 minutes. Pay special attention to sections between the fingers, under the nails, and on the surface of the palm. Be careful to imprint fingers before washing always on one half of a petri dish for comparison.
4. On petri dish 3, press your fingertips, which have been disinfected with a disinfectant by applying some 5ml of the latter on the palms and rubbing them until dry (special attention should again be paid to sections between fingers, under the nails and on the surface of the palm).
5. For petri dish 4, disinfect your hands using 70% ethanol by applying some 5ml of ethanol on the palms and rubbing them until dry, whereby special attention should again be paid to sections between fingers, under the nails and on the surface of the palm.
6. Incubate the petri dishes at a temperature between 25 and 37°C for several days (if possible at 37°C, as that temperature provides the maximum growth of microorganisms).

Microorganisms in the air and on everyday objects:

1. Mark a petri dish with agar, open it and speak into the open petri dish for 5 minutes at a distance of 10cm, and then cover it back.
2. Open petri dish 2 with agar and cough or sneeze into it.
3. Leave petri dish 3 open for 10 minutes.
4. Mark a petri dish for each selected surface or object (cell phone, lipstick, toilet pan, door handle, floor, money, etc.). Dip a swab in saline solution, swab the selected surface, and then spread it over a petri dish.
5. Incubate all petri dishes at a temperature between 25 and 37°C for several days.

Testing the effectiveness of natural antibiotics:

1. Mark a petri dish with agar that you have already infected during pouring (the agar was not sterilised and no aseptic work was done) and place or drop the selected antibiotic (garlic, ginger, honey, toothpaste, disinfectant, etc.) on the centre.
2. Incubate petri dishes at a temperature between 25 and 37°C for several days.
3. Check and measure the size of a possible zone of inhibition surrounding the slice or drop every day.

RESULTS:

Observe the experiment and describe what happens and why.

REPORT:

1. Observe growth on plates and compare the effectiveness of different ways of hand washing. Observe for the number of colonies and for different types of colonies. What can you conclude?

2. What can you conclude based on a comparison of the plates in which you spoke and coughed or sneezed? What about the dish you left open for 10 minutes?

3. Compare the agar plates with swabs from different everyday objects. Which have more and which less bacteria? Why?

4. Compare the measured zones of inhibition produced by different antibiotics. What can you conclude? Did any of them fail to produce a zone of inhibition?

TEST:

1. What is aseptic work?
2. How do you check the purity of a microbial culture?
3. How does temperature affect the growth of microorganisms? Illustrate it with a chart.
4. What is an antibiogram?
5. What is antibiotic resistance and what types of resistance are there?

EVALUATION OF THE PRACTICAL:

Knowledge for practical:			
Experimental exercise:			
Results and answers:			
Compliance with security rules:			
Review date:		Supervisor signature:	