Chemical Methods of Control

Objectives

1. Define the following terms: disinfectant, antiseptic, and antibiotic.
2. Perform a disk-diffusion test.
3. Evaluate the relative effectiveness of various chemical substances as antimicrobial agents.

Background

Wide varieties of chemicals called antimicrobial agents are available for controlling the growth of microbes. **Disinfectants** are chemical agents used on inanimate objects to lower the level of microbes on their surfaces; antiseptics are chemicals used on living tissue to decrease the number of microbes. Disinfectants and antiseptics affect bacteria in many ways. Those that result in bacterial death are called bactericidal agents. Those causing temporary inhibition of growth are bacteriostatic agents. No single chemical is the best to use in all situations. Antimicrobial agents must be matched to specific organisms and environmental conditions. Additional variables to consider in selecting an antimicrobial agent include pH, solubility, toxicity, organic material present, and cost. In evaluating the effectiveness of antimicrobial agents, the concentration, length of contact, and whether it is lethal (*-cidal*) or inhibiting (*-static*) are the important criteria.

The observation that some microbes inhibited the growth of others was made as early as 1874. Pasteur and others observed that infecting an animal with *Pseudomonas aeruginosa* protected the animal against *Bacillus anthracis.* Later investigators coined the word antibiosis (against life) for this inhibition and called the inhibiting substance an antibiotic, a substance produced by a microorganism that inhibits other microorganisms. In 1928, Alexander Fleming observed antibiosis around a *Penicillium* mold growth on a culture of staphylococci. He found that culture filtrates of *Penicillium* inhibited the growth of many gram-positive cocci and *Neisseria* spp. In 1940, Selman A. Waksman isolated the antibiotic streptomycin, produced by an actinomycete. This antibiotic was effective against many bacteria that penicillin did not affect. Actinomycetes remain an important source of antibiotics. *Streptomyces* bacteria produce nearly 70% of all antibiotics. Today, research investigators look for antibiotic-producing actinomycetes and fungi in soil and antimicrobial compounds made by plants and animals. Antimicrobial chemicals used internally, whether natural (**antibiotics**) or synthetic, are called antimicrobial drugs. To treat an infectious disease, a physician or dentist needs to select the correct antimicrobial agent intelligently and administer the appropriate dose; then the practitioner must follow that treatment to be aware of resistant forms of the organism that might occur. The clinical laboratory isolates the pathogen (disease-causing organism) from a clinical sample and determines its sensitivity to antimicrobial agents.

In the disk-diffusion method, a Petri plate containing an agar growth medium is inoculated uniformly over its entire surface. Paper disks impregnated with various antimicrobial agents are placed on the surface of the agar. During incubation, the antimicrobial agent *diffuses* from the disk, from an area of high concentration to an area of lower concentration. An effective agent will inhibit bacterial growth, and measurements can be made of the size of the zones of inhibition around the disks. The zone size is affected by such factors as the diffusion rate of the antimicrobial

agent and the growth rate of the organism. Larger zones of inhibition indicate that a *low* concentration of the chemical stops growth. We will use the effectiveness scale shown in Table 1.

Table 1. Evaluating disinfectants and antiseptics

Effectiveness	Zone of inhibition
Highly	≥23 mm
Moderately	11 - 22 mm
Low-level	≤10 mm

To minimize the variance between laboratories, the standardized Kirby-Bauer test for agar diffusion methods is performed in many clinical laboratories with strict quality controls. This test uses *Mueller-Hinton agar*. Mueller-Hinton agar allows the antimicrobial agent to diffuse freely. For evaluation of antimicrobial agents, three strains of bacteria are used in this test: *Escherichia coli*, *Staphylococcus aureus,* and *Pseudomonas aeruginosa.*

Materials

FIRST PERIOD

Petri plate containing Mueller-Hinton agar
Sterile cotton swab
Forceps
Alcohol
Test substance: chemical agents such as bathroom cleaner, floor cleaner, mouthwash, lens cleaner, and acne cream. **Bring your own.**

SECOND PERIOD

Ruler

Procedures

FIRST PERIOD

CAUTION: *Be careful* not to spill or splash the bacterial cultures. THE TUBES MUST BE KEPT UPRIGHT. If you spill any of the cultures, cover the spill with paper towels and Sanisol. Allow it to soak for 5 min., then wipe up the liquid with clean towels.
WASH YOUR HANDS BEFORE AND AFTER THIS EXERCISE.

1. Aseptically swab the assigned culture onto the appropriate plate. Swab in three directions to ensure complete plate coverage (**Figure 1**). Why is complete coverage essential? _____ Let stand at least 5 minutes.

2. Sterilize forceps by dipping them in alcohol and burning off the alcohol.

CAUTION: While they are burning, hold the forceps pointed down. Keep the beaker of alcohol away from the flame.

   Obtain a filter paper disk and saturate it with an antimicrobial agent. Blot the disk to remove excess liquid. Place the disk on the surface of the agar (**Figure 2a**). Gently tap the disk with the forceps to ensure better contact with the agar. Write "1" on the underside of the Petri plate Repeat, beneath this disk.

3. Repeat the step 2 placing five different disks the same distance apart on the Petri plate. See the location of the disks in **Figure 2b**. Record the agents and the corresponding number in your Laboratory Report.

4. Incubate the plate, inverted, at 35°C 24-48 hr.

SECOND PERIOD

1. Measure the zones of inhibition in millimeters, using a ruler on the underside of the plate (see Figure 25.3b). If the diameter is difficult to measure, measure the radius from the center of the disk to the edge of the zone. Multiply the radius by 2 to get the diameter of the zone.

2. Record the zone size and, indicate whether the organism is susceptible, intermediate, or resistant. Record the results of students using the other two bacteria. Colonies within a zone of inhibition are resistant to that antibiotic.

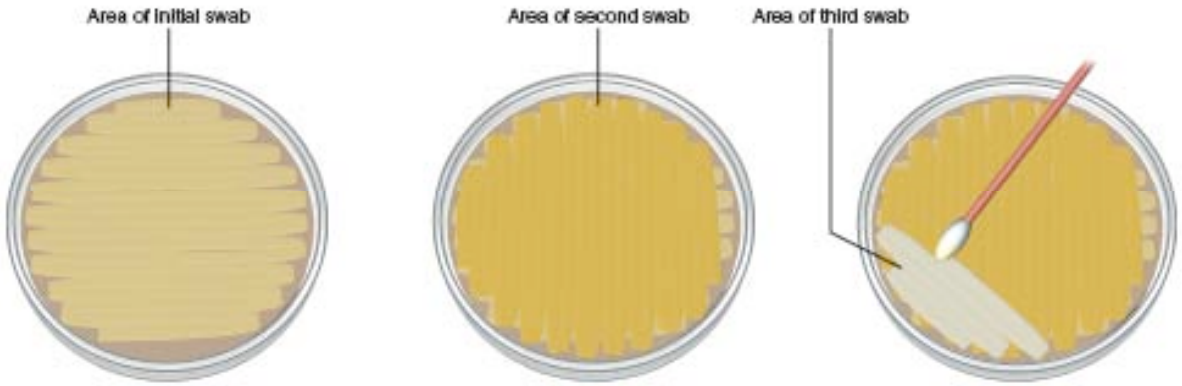

Figure 1. Inoculating the Mueller-Hinton agar plate. Dip a cotton swab in the culture to be tested, and swab across the surface of the agar *without leaving any gaps*. Using the same swab, swab the agar in a direction perpendicular to the first inoculum. Repeat, swabbing the agar at a 45° angle to the first inoculum.

(a) Place disks impregnated with antimicrobial agents on an inoculated culture medium with sterile forceps to get the pattern shown in **(b)**.

(b) After incubation, measure the diameters of zones of inhibition.

Figure 2. Disk-diffusion method.

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Name_____
Date _____

Hypotheses

1. All antimicrobics will be equally effective against gram-positive and gram-negative bacteria. *Agree/disagree*

2. All antimicrobics will be equally effective the gram-negative *E. coli* and *P. aeruginosa* bacteria. *Agree/disagree*

Results

Antimicrobial agent	*S. aureus*		*E. coli*		*P. aeruginosa*	
	Zone (mm)	S, I, R*	Zone (mm)	S, I, R*	Zone (mm)	S, I, R*

*Sensitive, intermediate, resistant

Conclusions

1. Do you accept or reject your hypotheses?

2. Which antimicrobial agents were most effective against each organism?

3. Why isn't one antimicrobial agent equally effective against all three bacteria?

Questions

1. Is the disk-diffusion technique measuring bacteriostatic or bactericidal activity? Briefly explain.

2. Read the label of the preparations you tested. What is (are) the active ingredient(s)?