Chemical Methods of Control: Antimicrobial Drugs

The aim of medicine is to prevent disease and prolong life; the ideal of medicine is to eliminate the need of a physician.

WILLIAM JAMES MAYO

Objectives

After completing this exercise, you should be able to:

1. Define the following terms: antibiotic, antimicrobial agent, and MIC.
2. Perform an antibiotic sensitivity test.
3. Provide the rationale for the agar diffusion technique.

Background

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.** 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 were not affected by penicillin. Actinomycetes remain an important source of antibiotics. Today, research investigators look for antibiotic-producing actinomycetes and fungi in soil and have synthesized many antimicrobial substances in the laboratory. Antimicrobial chemicals absorbed or used internally, whether natural (antibiotics) or synthetic, are called **antimicrobial agents.**

A physician or dentist needs to select the correct antimicrobial agent intelligently and administer the appropriate dose in order to treat an infectious disease; then the practitioner must follow that treatment in order 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 concentration of antimicrobial agent at the edge of the zone of inhibition represents its **minimum inhibitory concentration (MIC).** The MIC is determined by comparing the zone of inhibition with MIC values in a standard table (Table 25.1). The MIC values are determined by doing a broth dilution test in a laboratory by using a test bacterium. The zone size is affected by such factors as the diffusion rate of the antimicrobial agent and the growth rate of the organism. 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.

In this exercise, we will evaluate antimicrobial agents by the disk-diffusion method.

Materials

Petri plate containing Mueller-Hinton agar

Sterile cotton swab

Dispenser and antimicrobial disks

Forceps

Alcohol

Ruler (second period)

Cultures (as assigned)

Staphylococcus aureus broth

Escherichia coli broth

Pseudomonas aeruginosa broth

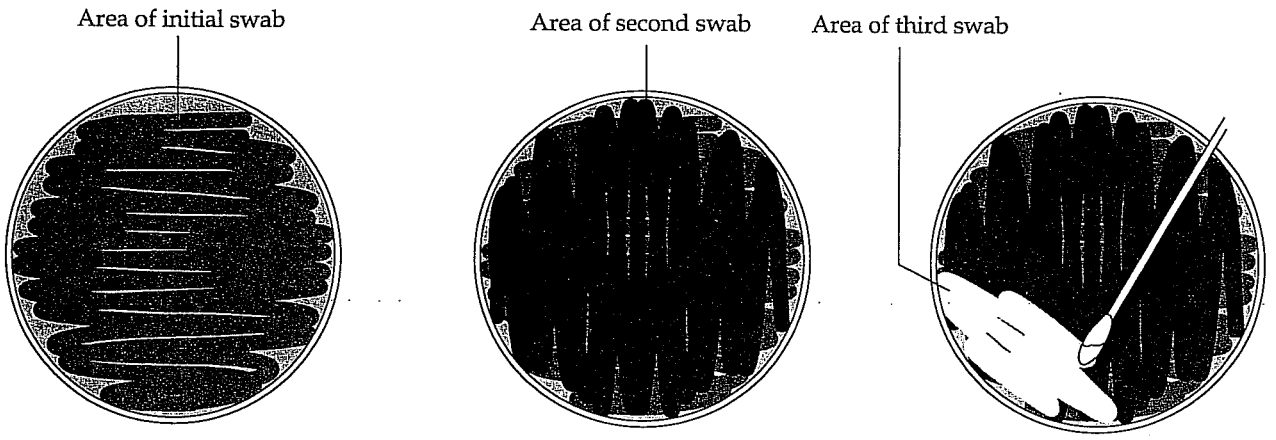


Figure 25.1

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.

Techniques Required

Inoculating loop technique, Exercise 10

Aseptic technique, Exercise 10

Procedure

1. Aseptically swab the assigned culture onto the appropriate plate. Swab in three directions to ensure complete plate coverage (Figure 25.1). Why is complete coverage essential? _____

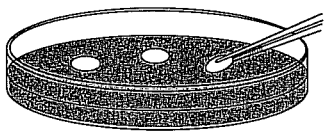
_____

Let stand at least 5 minutes.

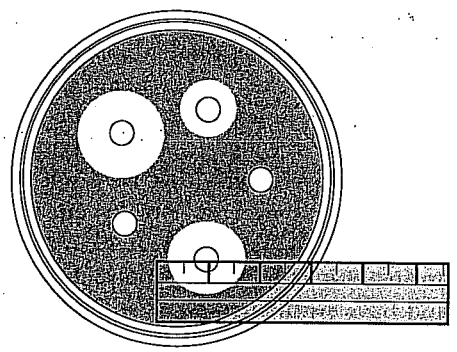
2. Follow procedure a or b.
   a. Place the antimicrobial-impregnated disks by pushing the dispenser over the agar. Sterilize your loop and touch each disk with the sterile inoculating loop to ensure better contact with the agar. Record the agents and the disk codes in your Laboratory Report. Circle the corresponding chemicals in Table 25.1.
   b. Sterilize forceps by dipping them in alcohol and burning off the alcohol.

> ⚠ **While it is burning, hold the forceps pointed down. Keep the beaker of alcohol away from the flame.**

Obtain a disk impregnated with a antimicrobial agent and place it on the surface of the agar (Figure 25.2a). Gently tap the disk with the forceps to ensure better contact with the agar. Repeat, placing five to six different disks the same distance apart on the Petri plate. See the location of the disks in Figure 25.2b. Record the agents and the disk codes in your



(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 25.2

Disk-diffusion method.

Table 25.1

Interpretation of Inhibition Zones of Test Cultures

Disk Symbol	Antimicrobial Agent	Disk Content	Diameter of Zones of Inhibition (mm)		
			Resistant	Intermediate	Susceptible
AM	Ampicillin when testing gram-negative bacteria	10 µg	<13	14–16	>17
	Ampicillin when testing gram-positive bacteria	10 µg	<28	—	>29
C	Chloramphenicol	30 µg	<12	13–17	>18
CAZ	Ceftazidime	30 µg	<14	15–17	>18
CB	Carbenicillin	100 µg	<19	—	>23
	Carbenicillin when testing *Pseudomonas*	100 µg	<13	—	>17
CF	Cephalothin	30 µg	<14	—	>18
CIP	Ciprofloxacin	5 µg	<15	16–20	>21
E	Erythromycin	15 µg	<13	14–22	>23
Fox	Cefoxitin (Mefoxin)	30 µg	<14	—	>18
G	Sulfisoxazole (Gantrisin)	300 µg	<12	13–16	>17
GM	Gentamicin	10 µg	<12	13–14	>15
IPM	Imipenem	10 µg	<13	14–15	>16
P	Penicillin G when testing staphylococci	10 units	<28	—	>29
	Penicillin G when testing other bacteria	10 units	<14	—	>15
R	Rifampin	5 µg	<16	17–19	>20
S	Streptomycin	10 µg	<11	12–14	>15
SxT	Trimethoprim/ Sulfamethoxazole	1.25 µg/ 23.75 µg	<10	11–15	>16
Te	Tetracycline	30 µg	<14	15–18	>19
VA	Vancomycin	30 µg	<9	10–11	>12
	Vancomycin when testing enterococci	30 µg	<14	15–16	>17

Source: National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk Susceptibility Tests.

Laboratory Report. Circle the corresponding chemicals in Table 25.1.

3. Incubate the plate, inverted, at 35°C until the next period. Measure the zones of inhibition in millimeters, using a ruler on the underside of the plate (Figure 25.2b). If the diameter is difficult to measure, the radius from the center of the disk to the edge of the zone can be measured. Multiply the radius by 2 to get the diameter of the zone. Record the zone size and, based on the values in Table 25.1, indicate whether the organism is susceptible, intermediate, or resistant. Record the results of students using the other two bacteria. (See Color Plates VIII.1 and VIII.2.)

Exercise 25

Chemical Methods of Control: Antimicrobial Drugs

LABORATORY REPORT

NAME _____

DATE _____

LAB SECTION _____

Purpose

_____

_____

_____

Data

Antimicrobial Agent	Disk Code	*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*	
		Zone Size	S, I, or R*	Zone Size	S, I, or R*	Zone Size	S, I, or R*
1.							
2.							
3.							
4.							
5.							
6.							
7.							
8.							

*S = susceptible; I = intermediate; R = resistant.

Conclusions

Which antimicrobial agents were most effective against each organism? _____

_____

_____

Questions

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

_____

_____

2. In which growth phase is an organism most sensitive to an antimicrobial agent? _____

3. Why is the disk-diffusion technique not a perfect indication of how the drug will perform in vivo? What other factors are considered before using the antimicrobial agent in vivo? _____

_____

_____

4. Using your textbook or other references, match each of the antimicrobial agents listed in Table 25.1 with its type and method of action.

Type of Antimicrobial Agent

a. Aminoglycosides
b. β-lactams
c. Carbapenems
d. Cephalosporins
e. Glycopeptides
f. Macrolides
g. Monobactams
h. Quinolones
i. Sulfonamides
j. Tetracyclines
k. None of the above

Method of Action

1. Inhibit enzyme activity
2. Inhibit cell wall synthesis
3. Inhibit protein synthesis
4. Inhibit nucleic acid synthesis

	Type	Method of Action
Ampicillin		
Carbenicillin		
Cefoxitin		
Ceftazidime		
Cephalothin		
Chloramphenicol		
Ciprofloxacin		
Erythromycin		
Gentamicin		
Imipenem		
Penicillin G		
Rifampin		
Streptomycin		
Sulfisoxazole		
Tetracycline		
Trimethoprim/Sulfamethoxazole		
Vancomycin		

5. From your results, which type of antimicrobial agent was most effective against gram-negative bacteria?

_____

Against gram-positive bacteria? _____

Critical Thinking

1. What effect would the presence of tetracycline in the body have on penicillin therapy?

2. The following results were obtained from a disk-diffusion test against a bacterium:

Antibiotic	Zone of Inhibition (mm)
A	6
B	18
C	11
D	18

Which drug should be used to treat an infection caused by this bacterium? Briefly explain.

3. The broth dilution test can be used to determine the effectiveness of an antibiotic. In this test, serial dilutions of the antibiotic were set up in the wells of a microtiter plate. Equal amounts of broth culture of *Staphylococcus aureus* were added to each well. After incubation, the wells were examined for bacterial growth. Wells with no growth were subcultured in nutrient broth without the antibiotic. Results were recorded as (+) for growth and (−) for no growth.

Antibiotic	Dilution	Growth	Growth in Subculture
A	1:10 through 1:70	−	−
	1:80	−	−
	1:90	−	+
	1:100	−	+
	1:200 through 1:500	+	+
B	1:10 through 1:150	−	−
	1:160	−	+
	1:170	+	+
	1:180	+	+
	1:190 through 1:500	+	+