

# General Microbiology Laboratory

## Lab Session 5 - Antimicrobials

### Introduction

Microorganisms are susceptible to a wide variety of chemical agents. Antiseptics, disinfectants and antibiotics kill or inhibit the growth of microorganisms by interacting with microbial structures or their metabolic machinery. However, different microorganisms have different susceptibilities to different chemicals. And, different chemicals are inherently toxic to microorganisms at different doses. There are a number of routine methods that are designed to specifically evaluate the susceptibility of an organism to an antibiotic, or the effectiveness of an antimicrobial substance. The purpose of this lab is to determine the antimicrobial activity of a variety of materials using a standard, ultra low tech, disk diffusion assay. The Methods are dull, but the concepts to be learned are worth the effort.

#### A. Review the following for reference:

1. Lab Manual: Exercise #24- Page 147. Read "Background" only.  
Exercise #25 - Pages 153 to 155. Note especially Fig. 25.1.
2. Text: Chapter 7 – Review, especially section 7.1  
Chapter 34 – Review, especially section 34.3.
3. Pay particular attention to the following:
  - Methods used to determine the sensitivity of microorganisms to antimicrobials
  - Disk diffusion techniques.

#### B. Do the following during lab:

1. Work in 3 groups of 6 students to collect all the data needed.
2. Each individual will need one broth culture of one of the following m.o.'s:
  - Staphylococcus aureus*\* - A gram positive coccus from human nasopharynx.
  - Escherichia coli* - A gram negative rod from the human gi tract.
  - Enterococcus faecalis*\* - A gram positive coccus from the human gi tract.
  - Pseudomonas aeruginosa*\* – A gram negative rod from human tissues.
  - Candida albicans* – A yeast from oral and vaginal mucous membranes
  - C. xerosis* – A gram positive coccobacillary mo from human tissuesAND
  - Unknown*\* - Each individual will need the broth culture from your throat isolate.  
You need to know the description of the mo (i.e. gram stain & morphology)

\*Note: These mo's are BSL-2 microorganisms and must be handled with caution!

3. Prepare four "lawns of growth" for each of your 2 m.o.'s using Trypticase Soy Agar. (See C.1.)

4. For each organism or your unknown, add to the lawns of growth the following: (See C.2.)

#### Plate 1 - Antiseptics

1A = Listerine (Thymol, .064% & eucalyptol, .092%)  
1B = Merthiolate (0.1%, aq.)  
1C = Betadine (1% available iodine, aq)  
1D = Scope (Cetylpyridinium chloride, ?)  
1E = Bac Down Hand soap (0.1% Triclosan, aq)

#### Plate 2 - Disinfectants

2A = Lysol (1.2%, Benzyl ammonium chloride)  
2B = Hydrogen peroxide (3%, aq)  
2C = Ethanol (70%, aq)  
2D = Bac Down Disinfectant (7% BAC)  
2E = Bleach (6% hypochlorite, aq)

#### Plate 3 - Antibiotics

3A = Erythromycin  
3B = Tetracycline  
3C = Polymyxin B  
3D = S x T  
3E = Rifampin

#### Plate 4 - Antibiotics

4A = Penicillin  
4B = Ampicillin  
4C = Ciprofloxacin  
4D = Gentamycin  
4E = Cefaclor

5. Incubate plates at 37 C for 24-48 hours.

### **C. Techniques**

1. Lawn of growth/spread plate: a) Use a sterile swab to  
b) inoculate the entire surface of an agar plate.
2. Antimicrobial: a) Carefully impregnate a sterile  
paper disk with the necessary chemical  
and transfer it to the agar plate using forceps, or  
b) Use a disk transfer device, if available.
3. Zone of Inhibition: Use a ruler to measure the  
inhibition of growth (diameter, in mm).

### **D. Results**

1. Measure any zone of inhibition (diameter in mm) around the disks (See C.3.).
2. Prepare a table of results for your notebook. You will need to share data on the other supplied microorganisms. You do not need your partner's data on their unknowns.

<b>Treatment</b>	<i>Staph.. aureus</i>	<i>E. coli</i>	<i>Pseud. aerug</i>	<i>Entero.. faecalis</i>	<i>Candida albicans</i>	<i>Coryne. xerosis</i>	Unknown
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Plate 1-Antiseptics

1A = Listerine mouthwash

1B = Merthiolate

1C = Betadine

1D = Scope mouthwash

1E = Bac Down hand soap

Plate 2-Disinfectants

2A = Lysol

2B = Hydrogen peroxide

2C = Ethanol

2D = Bac Down disinfectant

2E = Bleach

Plate 3-Antibiotics

3A = Erythromycin

3B = Tetracycline

3C = Polymixin B

3D = S x T

3E = Rifampin

Plate 4-Antibiotics

4A = Penicillin

4B = Ampicillin

4C = Ciprofloxacin

4D = Gentamycin

4E = Cefaclor

## **E. Discussion and Conclusions**