EXPERIMENT 6

Antimicrobials: Control of Bacterial Growth

INTRODUCTION

Infectious agents on environmental surfaces, given the correct circumstances, may potentially find their way into an unsuspecting victim. Thus, it is important to keep the surfaces we regularly come in contact with properly disinfected. In the medical, clinical, and laboratory setting working with infectious agents is risky business and proper sterilization and sanitization procedures are paramount. **Sanitization** refers to the use of chemical disinfectants onto hard, non-porous surfaces in order to eliminate *most* bacteria. Sanitization does not remove all bacteria especially bacteria that produce resistant spores. On the other hand, **sterilization** is the complete destruction of all living microorganisms, viruses, and reproducible spores through various means. Sanitization and sterilization are employed by *chemical* or *physical* means. Physical agents for the control of microbial growth include heat (dry, moist, flaming, pasteurization), freezing, filtration, drying, and irradiation (UV). Chemical agents include antimicrobial substances, preservatives, heavy metals, antiseptics, and disinfectants.

Chemical agents that kill or inhibits microbial growth may be classified as disinfectants, antiseptics, or antibiotics. **Antibiotics** are molecules produced and secreted naturally by one organism in order to combat other microorganisms. Antibiotics that effectively *kill* off bacteria are termed **Bactericidal** and those that *inhibit* bacterial growth are **Bacteriostatic**. **Antiseptics** and **disinfectants** are commercially formulated chemicals that differ in that antiseptics are formulated to be nontoxic when exposed to skin and mucosal surfaces for a period of time while disinfectants are used only on hard, non-porous environmental surfaces and are toxic to exposed mucosal surfaces.

Different agents have varying effects on different bacteria. In other words, the rate and efficacy of a chemical or physical agent for sterilization or sanitization varies with the agent used and the type of microbe. Also, whether an agent is bacteriostatic or bactericidal is dependent on its *concentration* and *duration* (*time*) of contact with the target microorganism. The metabolic state of the target microbe can also affect the efficiency of an agent: dormant cells and spores are more difficult to remove than vegetative cells. Few other factors that influence the action

of agents are concentration and type of chemical, time of contact, microbe type, cell concentration, and temperature.

The mode of action of antibiotics differs from those of antiseptics and disinfectants. **Mode of action** refers to the biochemical interaction through which a drug substance produces its pharmacological effect. Antibiotics act on microbes usually by changing an essential metabolic pathway, such as amino acid, protein, or cell wall synthesis. Table 1 demonstrates the different mode of action of various antibiotics we will be using in the lab. Conversely, the mode of action of antiseptics and disinfectants is mainly by denaturing proteins, dissolving cytoplasmic membranes, and oxidizing.

An important assay used to test the potency and efficacy of antibiotics and antiseptics and other antimicrobial substance is done by using a filter paper disc soaked with the substance to be tested and exposing the target microbe atop an agar surface. This paper disc-agar diffusion method is done by first spreading the bacteria to be tested for susceptibility onto the agar plate in order to form an even bacterial "lawn" (Appendix Figure 10). After spreading the organism, paper filter discs containing either the test antibiotics or dipped in antiseptics are placed on the agar surface. The plate is then incubated and after incubation the plates are examined for the **zone of inhibition** (area of no growth) around the disks (Figure 1).

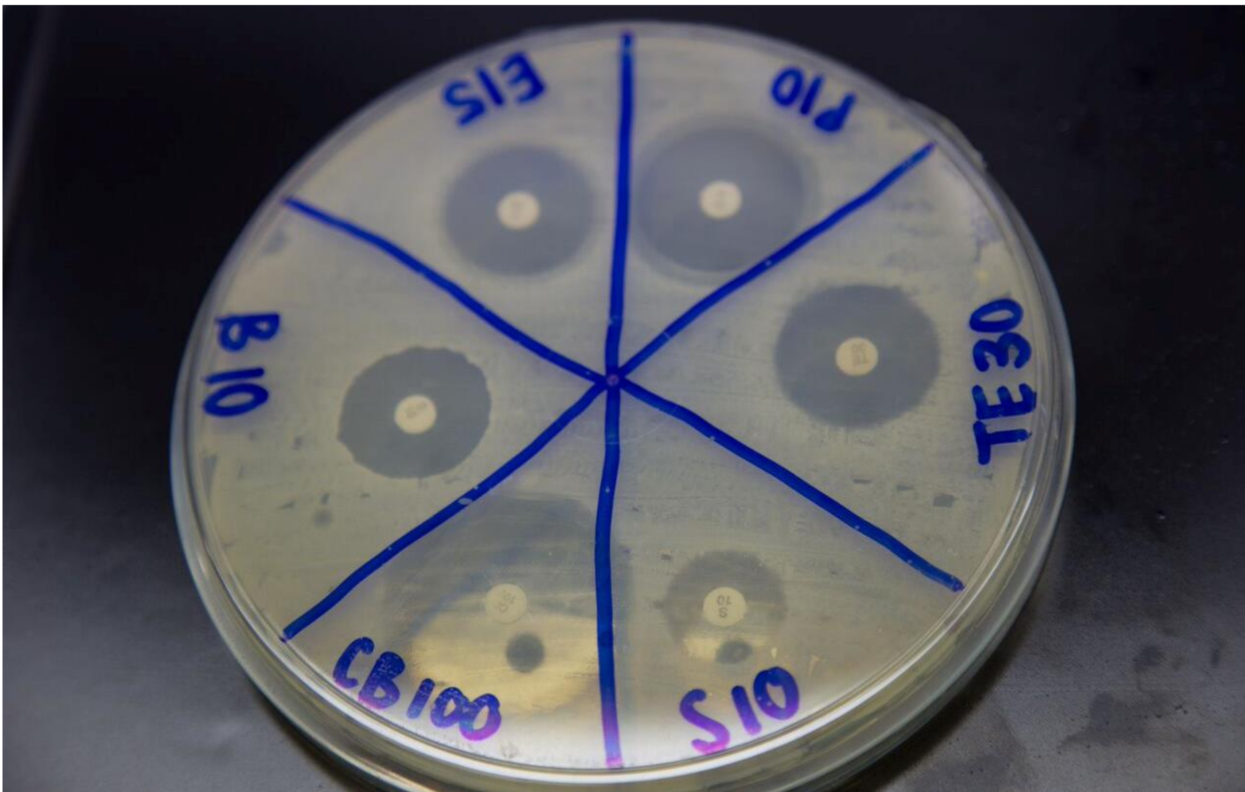

Figure 1. Mueller Hinton Agar plate inoculated with E. coli and subjected to 6 different antibiotics showing zones of inhibition.

Materials:

1. **Antiseptics: 5 different commercial mouthwashes**
2. **Antibiotics: antibiotics discs inside dispensers**
3. **(2) BHI Agar plates for antiseptic assay**
4. **(2) Mueller-Hinton agar plates for antibiotic assay**
5. **(1) Large Nutrient agar plate**
6. **(1) sterile capped tube with saline solution**
7. **(1) BHI broth tube (1ml)**
8. **Sterile cotton swabs**
9. **Forceps dipped in beaker with 95% ethanol**
10. **Sterile disc paper**
11. **Onion, pepper seeds, garlic, and three other food items**
12. **Numbered bacteria in BHI broth tube**

Procedures:

Part I. Antiseptic Assay

1. With a sterile swab, swab your teeth. Place the swab into the BHI broth tube (0.5ml), snap off the end of the swab by breaking it and place the tube cap on. Incubate at 37°C for two hours.

2. **Label** your 2 BHI plates plate as shown in Figure 1 into six equal sections. Label one plate with the mouth bacteria and the other with the test bacteria. Label each section with the antiseptics you will be testing.

3. After two hours, swab the surface of one BHI plate with the mouth bacteria broth. Swab the other BHI plate with the known test bacteria. Swab the plate according to the standard procedures described in section (Appendix Figure 10). Allow the plate to air dry.

4. With the alcohol sterilized forceps, take a sterile paper disc and insert it into one of the commercial washes, remove any excess liquid by pressing onto side of cup, and place it on the swabbed plate (Figure 1). Repeat this with remaining mouthwashes.

5. Incubate both plates at the temperature indicated by your TA for 48 hours. Next lab you will measure the size of the zones of inhibition in millimeters (mm) and record in your worksheet.

Part II. Antibiotic Assay

1. **Label** your 2 MH (Mueller Hinton) plates into six equal parts as above. Swab your known test bacteria on one plate and your bacteria from your RODAC plate in Experiment 5.

2. After swabbing both plates, dispense the antibiotic discs onto the surfaces using sterile forceps to place evenly and to set disc firmly onto agar surface.

3. Incubate both plates at the temperature indicated by your TA for 48 hours. Next lab you will measure the size of the zones of inhibition (if any) in millimeters (mm) and record in your worksheet.

Part III. Bioactivity of Food

1. Label your Large Nutrient Agar plates into six equal parts as above. Swab your known test bacteria onto the plate as described above.

2. After swabbing the nutrient agar plate, place the various food items made available. Use sterile forceps to maneuver the food items gently onto the agar surface.

3. Incubate the plate at 30 or 37°C for 48 hours. Next week, measure the size of the zones of inhibition in millimeters (mm) if any and record your observations in your worksheet.

Bacterial ID________________

Part I & II Antiseptic and Antibiotic Assay
Directions: Insert the antiseptic and antibiotic with corresponding zones of inhibitions for both bacteria samples in millimeters (mm).

Antiseptic	**Known Bacteria**	**Mouth Bacteria**
1.		
2.		
3.		
4.		
5.		
6.		

Antibiotic	**Known Bacteria**	**Experiment 5**
1.		
2.		
3.		
4.		
5.		
6.		

Part III Bioactivity of Food

Directions: Place the zone of inhibition (if any) in response to the six food products including onion, garlic, and pepper in millimeters (mm).

Food Product	Zone of Inhibition (mm)
1.	
2.	
3.	
4.	
5.	
6.	

Part IV. Questions

Directions: Answer the following questions using your experimental data.

1. Which antiseptic was the most effective against each individual bacterium tested? Which antiseptic was the least effective?

2. Which antibiotic was the most effective against the bacteria from the Bacteria Transmission lab and the Known Bacterial Sample given?

3. Besides the zone of inhibition, what other factors (mode of action, toxicity, target organisms…) would you consider if prescribing an antibiotic to a patient infected by known bacteria?

4. Which of the food products was the most effective against the bacteria tested? Is the action of the food item bacteriostatic or bactericidal? How can you prove a compound is bacteriostatic?

5. How can the information obtained from the food bioactivity assay be applied or be useful into your daily life?