

EFFECTS OF CHEMICAL ANTIMICROBIALS ON MICROORGANISMS

I. OBJECTIVES

- To examine the effectiveness of various antiseptics and disinfectants on bacterial growth.

II. INTRODUCTION

Agents that prevent the growth of or kill microorganisms are called antimicrobial agents. These include heat, chemicals, UV light, etc. In this lab period, we will concentrate on chemical agents that are commonly used in industry, homes, laboratory, etc.

Microorganisms vary in their sensitivity to particular chemical agents. Antimicrobial agents block active metabolism and prevent the synthesis of macromolecules needed for reproduction. Since in dormant stages (e.g. the spore stage) the bacteria are not reproducing, the antimicrobial agents are not very effective against them.

One way to classify the chemical antimicrobials is according to their action (i.e., whether they kill or inhibit growth of the microorganisms). In this classification, we have germicides and microstatic agents.

1. Germicides: These are chemicals that kill growing microorganisms upon contact and are further classified according to their specificity as bacteriocides (kill bacteria), viricides (kill viruses), algaecides (kill algae) and fungicides (kill fungi).

Note: antibiotics are bacteriocides produced by living organisms.

2. Microstatics: These are chemicals that do not kill but inhibit the growth of microorganisms. The microorganisms are able to resume growth as soon as the effect of the microstatic agent is removed.

Another way to classify the chemical antimicrobials is according to their use. In this way, we can have antiseptics and disinfectants.

1. Antiseptics: These are chemicals that can be topically applied to biological tissue but are not necessarily safe for consumption. The most widely used antiseptic is alcohol, which is safe enough to be used on the skin around a wound. It is also used to disinfect contaminated objects. Alcohol dehydrates the cell, denatures proteins and extracts membrane lipids. Another common antiseptic is iodine, which kills all bacteria as well as spores.

- Disinfectants: These are chemicals that are used only to control the growth of organisms on inanimate objects and are not safe for medical use or consumption. Disinfectants can be either germicidal or microstatic in their effect. Any agent that oxidizes biological macromolecules can be a good disinfectant; for example, most of the household cleaning agents such as ammonia and bleach (sodium hypochloride) are disinfectants.

III. LABORATORY SUPPLIES

Cultures of <i>E. coli</i> and <i>Staph. epidermidis</i> , 10 ml/tt	4/lab
TSA plates	2/tablesides
Various antimicrobial agents, each 5 ml/tt	1/lab
Phenol, 2%, 5 ml/tt	4/lab
Ethyl alcohol, 70%, 5 ml/tt	4/lab
Sterile filter-paper	8/student
Forceps	4/lab
Sterile swabs	2/student

IV. PROCEDURE

Note: The students at each tableside will work together as a group.

- Dip a sterile swab into the *E. coli* culture provided and inoculate one TSA plate by streaking. Rotate the plate 90 degrees and re-swab. Repeat using a new TSA plate, a new sterile swab and the *Staph. epidermidis* culture.
- Draw lines on the back of each plate to divide it into 4 quadrants. Using sterile forceps, dip two filter paper disks into the phenol solution and place on one of the quadrants of each of the two plates. Repeat this step using 70% alcohol.
- Again repeat with 2 other antimicrobials from the list below. Label the 4 quadrants carefully and incubate plates at 30°C for 48 hours.

Compound	Use	Compound	Use
Formaldehyde, 1%	General preservative	Backdown, 3%	Disinfectant
Acetic Acid, 10%	Food preservative	Lysol	Disinfectant
Iodine, 2%	Topical antiseptic	Mercuric chloride, 1%	Disinfectant
Silver nitrate, 2.5%	Topical antiseptic	SDS, 1%	Disinfectant

- After incubation, examine your plates and note the halo of no growth or zone of inhibition around the disks. The size of the halo is an indication of the effectiveness of the chemical used in controlling the growth of the organisms. Measure and record the diameter of zones of inhibition to closest millimeter. Compare the effects of the antimicrobials on the two microorganisms used.

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Results of the Antimicrobial Exercise

NAME _____ DATE _____ GROUP NAME _____

PARTNER(S) _____

Diameter of zone of inhibition (mm)

Antimicrobial Agent

*E. coli**Staph. epidermidis*

1.

2.

3.

4.

Based on the results above, which antimicrobial agent would you recommend for control of each of the two species used? Discuss your results.