Microbiology Activity #5 - Control of microbial growth by radiation and antibiotics.

Microorganisms can be inhibited or killed by a variety of chemical agents and by chemical changes in their physical environment. This experiment examines the effectiveness of various antibiotics on bacterial growth, the use of disinfectants and antiseptics to control bacterial populations and the lethal effect of ultraviolet light. The control of microorganisms is critical in the treatment of infectious disease. Identification of effective antibiotics and their proper dosage are of utmost clinical significance.

Evaluation of disinfectants used to kill or inhibit microorganisms on inanimate objects is important in order to prevent accidental infection from a contaminated instrument or surface. Antiseptics are in widespread use to prevent the proliferation of microorganisms on tissue surfaces. These chemicals can either kill or inhibit microorganisms, but generally are not as toxic as disinfectants since antiseptics are normally applied to tissue surfaces. Biocides are chemical agents used to kill bacteria in commercial products (e.g. cosmetics) or in industrial systems (e.g., cooling towers). They are evaluated in a manner similar to that used for disinfectants.

Ultraviolet light is lethal to microorganisms, but the main drawback is that it is not able to penetrate some substances, such as glass and turbid water. UV radiation is used in biological safety cabinets, and in rooms to sterilize air. Commercial UV units are installed for water treatment to kill microorganisms that may be present in water. Small units are available to purify drinking water and are usually mounted under sinks. Industrial UV systems are used to treat large volumes of water that have become contaminated in manufacturing processes. The intensity and wavelength of light, time of exposure, and thickness of water are important considerations in any application where UV light is used to control microorganisms in water.

Chemicals used in the treatment of disease are called chemotherapeutic agents. Antibiotics historically were defined as chemicals produced by specific microorganisms that would inhibit or kill other microorganisms.

Antibiotics are now produced in a number of ways:

1. Naturally, through the action of a bacterial or fungal species.
2. Semi-synthetically, by a combination of natural products with added chemical synthesis
3. Synthetically, through chemical synthesis alone.

Most antibiotics are produced or synthesized through the action of a small group of bacterial and fungal genera. *Penicillium* and *Cephalosporium* are the main fungal genera, with *Bacillus* and *Streptomyces* being the predominant bacterial genera involved.

Before administering an antibiotic, it is essential to check the susceptibility of the microorganism to the agent prescribed. There has been an emergence of significant antibiotic resistance among microorganisms. To minimize the selection and spread of resistant strains, care must be taken in the use and administration of antibiotics. Several methods are employed to measure the sensitivity of bacteria to antibiotics; the one most commonly used is the filter-paper disk **or Kirby Bauer disk diffusion assay**. In this method an organism is distributed evenly over the surface of an agar plate. Then, filter-paper disks impregnated with the appropriate antibiotics are placed on the agar surface. The impregnated disks are commercially available and greatly facilitate testing.

Chemical agents have been used for centuries to control the growth of unwanted bacteria. Chemicals used to disinfect inanimate objects such as laboratory benches, restroom plumbing, or other large pieces of equipment are called **disinfectants.** These agents are usually too toxic to be used on human tissue. The agents that are safe to use on living tissue are referred to as **antiseptics.** Many commercially available agents are advertised with promotions such as “kills germs on contact,” and “get rid of unwanted germs.” Can these claims be substantiated? There is some terminology that has to be clarified before proceeding further. Some chemicals are capable of killing “germs” and are sometimes referred to as germicides. Their action is called **bactericidal** or viricidal, since bacterial death and viral inactivation actually occur. Many antiseptics are bacteriostatic, that is, they do not kill bacteria directly, but prevent the further multiplication of organisms. There are many tests designed to measure the effectiveness of a bactericidal or bacteriostatic agent. In this experiment, procedures to measure both the effectiveness of antiseptics and disinfectants are used.

Ultraviolet radiation causes microbial mutations - sudden, inheritable or lethal changes in the genetic material of microorganisms. After exposure to visible light: some of the damage is reversed. This process, which shields cells from the full antimicrobial impact of ultraviolet radiation, is called photoreactivation. In this exercise we demonstrate the lethal effects of ultraviolet radiation. We also examine the effects of light-activated deoxyribonucleic acid (DNA) repair mechanisms.

The bactericidal (able to kill bacteria) action of ultraviolet radiation is employed medically and commercially to reduce contamination. Lamps that emit ultraviolet radia-tion decrease the number of microbes in air. These lamps are employed in meat packing plants; their radiation lowers the incidence of spoilage caused by airborne fungi and bacteria. Ultraviolet lamps disinfect air in hospital operating rooms between operations. And, in microbial research laboratories, the risk of working with pathogens is reduced by decontaminating the air with ultraviolet radiation every time the microbiologist leaves the room.

By definition, ultraviolet light includes electromagnetic radiation with a wavelengths between 4 nm and 400 nm). Wavelengths in the range of 210 to 300 nm are those capable of producing the greatest lethal effects. It has been demonstrated that the peak lethality for most organisms is at 265 nm. The lethal action of ultraviolet light is due to its action on the DNA molecule. The radiation causes the formation of pyrimidine dimers, that is; the linking, via covalent bonds, of pyrimidine bases that lie next to one another on the same strand of DNA, and sometimes on opposite strands of the DNA molecule. The dimer that is formed cannot serve as a template for the DNA polymerase and, as a result, replication is prevented. The extent of the damage can be lethal for the organism unless repair takes place. There are a number of repair mechanisms that can occur. RecA protein is involved in an inducible repair system known as SOS repair, which acts rapidly, but is very error-prone and introduces mutations in the DNA. Where the damage is extensive, repair may not be possible, or else it is so error-prone that the exposure to ultraviolet radiation is lethal for the organism.

A. **Procedure - Antibiotic sensitivity testing**

1. Label and inoculate a tube of liquid medium for each of your known and unknown organisms. Incubate in the shaker to provide good aeration.

2. Obtain 8 plates of tryptic soy agar (TSA) or R2A agar, draw lines to divide each into 4 sections, and label the plates as follows, with the antibiotic names near the edges of each quadrant.

UK-XXX	amp carb amox pen	amp+ carb+ clav amox+ pen+	ery kan nal cm	rif str ctc tet
K-XXX	amp carb amox pen	amp+ carb+ clav amox+ pen+	ery kan nal cm	rif str ctc tet

2. Pipette 100 μL of the appropriate liquid cultures onto the plates (4 for each organism). Spread with a glass spreader that has been dipped in ethanol and passed through a flame.

3. Using four plates for each organism, arrange four or five sterile filter paper disks around the periphery of each plate

4. Pipette 5 μL of the appropriate antibiotics onto the disks. Incubate at room temp until the next lab meeting.

5. Measure the diameter of the zone of inhibition in millimeters around each antimicrobial agent and enter this data into the table below.

6. Considering the mechanism of action of each substance tested, do you notice any patterns of antibiotic sensitivity when comparing gram negative and gram positive organisms?

B. Procedure - Ultraviolet light sensitivity testing

1. Obtain a TSA or R2A plate, draw a line down the center of the plate, label one side for your known, one for your unknown, label the plate – UV exposure-TSA.

2. Dip a sterile swab in you liquid culture & spread on the appropriate location of the plate – do not streak for single colonies.

3. Bring the plate to the UV light source, remove the lid of the plate, cover two thirds of the plate (so one third of each culture is exposed) to the UV light. Allow the plate to be exposed for 45 seconds, then slide the index card so that only 1/3 is covered for the next 15 seconds.

4. Remove the plates from the UV light, cover, incubate at room temp for two days, and observe the pattern of growth and non-growth.

Antibiotic sensitivity results – Enter the zone of inhibition in mm

			known	unknown
antibiotic	ZOI	ampicillin (amp)(10ug)		
antibiotic	ZOI	amp(10ug) + clav		
antibiotic	ZOI	amoxicillin (amx)		
antibiotic	ZOI	amx + clav		
antibiotic	ZOI	Carbenicillin (carb) (100ug)		
antibiotic	ZOI	carb (100 ug) + clav		
antibiotic	ZOI	Penicillin (pen) (10u)		
antibiotic	ZOI	pen (10u) + clav		
antibiotic	ZOI	Clavulinic acid (clav)		
antibiotic	ZOI	Chloramphenicol (Cm) (30ug)		
antibiotic	ZOI	Chlortetracycline(Ctc) (30ug)		
antibiotic	ZOI	erythromycin (Ery)(15ug)		
antibiotic	ZOI	kanamycin (Kan)(30ug)		
antibiotic	ZOI	nalidixic acid (nal) (30ug)		
antibiotic	ZOI	Rifampicin(rif) (5ug)		
antibiotic	ZOI	streptomycin (str) (10ug)		
antibiotic	ZOI	tetracycline (tet)(30ug)		