Control of Bacterial Populations

I. Terminology:

sterilization - treatment to destroy all microbial life (even destroys bacterial endospores and fungal spores); there are no degrees of sterility, sterile is 100%! (i.e. – the autoclave)

disinfection (sanitation) - treatment to reduce the number of pathogens to a level at which they pose no danger of disease; **disinfectants** are used to kill microbes on inanimate objects (most disinfectants are too harsh for use on delicate tissue); most disinfectants do not kill spores. Some don't damage viruses. (Bleach, Alcohol)

antiseptic - kill microbes or inhibit their growth on skin or other living tissue; **antiseptics** are applied to living tissue. (Bactine, Betadine, Neosporin, Peroxide). Usually only topical (not internal). Most antiseptics claim to be "cidal" (see below)

sanitizer – typically used on food-handling equipment and eating utensils to reduce bacterial numbers so as to meet public health standards (may mean just washing with soap in some cases).

"-static" - treatments that inhibit rather than kill; ex. refrigeration. (bacteriostatic, fungistatic, etc.)

"-cidal" - treatments that kill. (bactericidal – kills any bacteria, fungicidal – kills fungus , viricidal – destroys virus particles, etc.) Germicidal is a more limited, targets only common pathogens.

chemotherapeutic agents - chemicals, incl. antibiotics, used to treat disease inside the body.

II. Physical Controls

A. Heat

1. simple, inexpensive, effective penetrates to kill microbes throughout the object; best method if material being treated is not damaged by heat. Kills by incineration or denatures proteins causing cell death.

2. Types

   a. **Dry Heat Sterilization** - ex. flaming loops, tubes in lab & hot air ovens (171°C, 1hr., 160°C for 2 hr., 121°C for 16 hrs.). Household oven cooking is this type.

   b. **Moist Heat Sterilization** - ex. boiling or in autoclaves; effective at a lower temperature than dry heat b/c it penetrates more quickly. Does not kill thermophiles, endospores. Autoclave is more effective than boiling- it uses pressure to raise the temperature above that of boiling (121°C, 15psi, for 20 min.)

   c. **Pasteurization** - limits growth, but does not sterilize; used to slow spoilage of milk & dairy products, wine, beer; advantage: causes minimal damage to the product; developed by Louis Pasteur;: heat to 63°C for 30 min. or 72°C for 15 sec.

B. Cold

1. Effect – "static" does not sterilize.

   a. **Refrigeration** - preserves food because it stops the growth of most species of microbes (slows chemical reactions);

   b. **Freezing** - kills most bacteria, but survivors can remain alive for long periods in the frozen state

C. Radiation

1. Two types of radiation that kill bacteria directly are **UV (ultraviolet) Light & Ionizing Radiation**. The effect of both is sterilization.

   a. **UV Light** – + inexpensive - kills only on surfaces. Can also be harmful to humans.

   b. **Ionizing Radiation** - technically complex; used only on industrial scale. Being used to sterilize some produce, much to the public's dismay. 1.) **X rays** 2.) **Gamma rays**

D. Membrane Filtration

1. Effect - physically removes cellular organisms (not viruses - they are too small).

2. Uses - in lab , used with media, antibiotics, & other heat sensitive materials; filtration is replacing pasteurization in some causes, because filtration causes even less damage; you may have heard of "cold filtered" beers.

E. Drying

1. **evaporation involving heat** - effect - kills many microbes; rarely used in lab because the high heat causes chemical changes (denaturation); is used in food industry.

2. **lyophilization** [freeze drying] - removes water directly sublimation (like dry ice); materials are frozen & placed in a vacuum chamber; avoids the chemical changes caused by heat drying; Used to make lab media (agar); used in food industry to make instant coffee, etc.; disadvantage - expensive.

III. Chemical Control

A. Mechanisms of Action (different ways to kill em)

1. **Affect Proteins** – The alteration of protein structure is called **denaturation.** Denaturation can be permanent (bacteriocidal) or temporary (normal structure can be restored – bacteriostatic). Mechanisms of denaturation include:

2. **Affect Membranes (proteins or lipids)**

3. **Affect Cell Wall Formation –** block newly divided cells from making new cell wall

4. **Affect Nucleic Acid Structure –** Damage DNA or block its use

5. **Affect Metabolism –** block enzyme functions, preventing cells from carrying out necessary activities.

C. Types of Germicides

1. **Surfactants (soap)** – Dissolves oils and surface dirt - wash away microbes, but do not kill them.

2. **Phenol & Phenolics (Lysol)** - - denature cell proteins, disrupt cell membranes. ill most organisms; action is not impaired by organic materials (remain active even in the presence of blood, feces, etc.)

3. **Alcohols (Ethanol & Isopropanol**)–dissolves lipids in cell membranes & denature proteins.

   Main disadvantage - does not sterilize skin because it evaporates quickly and does not penetrate deeply enough into skin pores. Do not kill endospores.

4. **Halogens** - inactivates enzymes by oxidation.

   1.) **Iodine** – antiseptic (**Betadine, Operand)**

   2.) **Chlorine / Bromine** –bleach; added to drinking water and swimming pools

5. **Hydrogen peroxide -** oxidizing agent (denatures proteins)

6. **Heavy Metals (**denature proteins)

   1.) **Mercurochrome** are used - basic first aid kit supplies for disinfecting skin & mucous membranes.

   2.) **Silver Nitrate** - once applied to eyes of newborns to prevent gonorrhea; the trend for a while was toward using antibiotics instead, but the development of antibiotic-resistant strains has necessitated the use of silver nitrate again.

   3.) **Selenium sulfide** – kills fungi, including spores; commonly used to treat fungal skin infections; included in dandruff shampoos (dandruff is often caused by a fungus).

7. **Alkylating Agents –** they alkylate (attach short chains of carbon atoms) to proteins and nucleic acids. (All are carcinogenic or toxic to humans).

   - **Formalin / formaldehyde** used to preserve tissues & to embalm; kills all microbes, including spores; lower concentrations are used to inactivate microbes for killed

vaccines.

- **Glutaraldehyde** - used to sterilize surgical instruments
- **Ethylene oxide** - gas; advantages: disappears from the object after treatment; disadvantage: **extremely toxic to humans** so must be used in a sealed chamber; kills all bacteria, including endospores; used to sterilize materials destroyed by heat (rubber gloves, animal feed, mattresses, first aide supplies, Petri dishes).

8. **Dyes –** Ex. Crystal violet blocks cell wall synthesis. It effectively inhibits growth of G(+) bacteria in cultures and in skin infections. It can be used to treat yeast infections.

III. Food Preservation

A. Temperature – See Heat under Physical Controls on Pg 1

B. pH - Acidity (low pH) prevents the growth of most microbes, especially in an anaerobic environment. Ex. adding vinegar (acetic acid) to foods.

C. Drying - drying & salting do not sterilize but preserve food by making it unable to support microbial growth for lack of water, an essential nutrient. (See pg. 1)

D. Chemicals - Various chemical preservatives are added to commercially prepared foods. Ex.:

1. **calcium propionate**, **sorbic acid**, **sodium benzoate** - all antifungal agents added to inhibit mold once a product has been opened.

5. **sodium nitrate (nitrite)** - antibacterial agent that prevents germination of Clostridium botulinum spores when added to bacon, ham, hot dogs.

IV. ANTIBACTERIAL AGENTS

A. Selective Toxicity – drug harms the microbe without causing significant damage to the host. When searching for ways to treat disease, scientists look for differences between the human (or animal) host and the pathogen. Ex. Penicillin interferes with cell wall synthesis. Animal cells have no cell walls, so penicillin is not toxic to animals.

B. Spectrum of Activity – the range of different microbes against which an antimicrobial agent acts. Example: **Broad spectrum**: G(+) and G(-) bacteria vs. **Narrow spectrum**: G(-) only;