CONTROL of MICROBES

- Sterilization and Disinfection - Ch 12
- Chemotherapeutics (Antimicrobial Therapy) – Ch 13
- Education and Information:
  - Disease Processes (Host-Microbe Relationships) – Ch 14
  - Epidemiology & Nosocomial Infections – Ch 15
- Nonspecific Host Defenses – Ch 16
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Principles of Sterilization and Disinfection:

- **Sterilization** – the killing or removal or all forms of a microbe in or on an object
  - Since “0” does not exist, Sterilization is considered complete when there is no greater than a 1/1,000,000 chance of finding a living form of a microbe
  - There are NO “degrees” of sterility
Principles of Sterilization and Disinfection:

- **DISINFECTION** – the reduction of pathogenic microbes on objects or other non-living material to levels deemed safe by public health authorities.

- **ANTISEPSIS** – the reduction of pathogenic microbes on mucous membranes or living tissue.

- **SANITIZATION** – the reduction of pathogenic microbes on food handling equipment to levels determined safe by a local Co. Dept. of Health.
Microbial Control

- Chemical Agents
- Physical Agents
General Rules of Chemical Control...

- Use at proper **temperature** and **concentration** (slightly more is OK and slightly warmer is OK – check for proper pH - usually at pH 6.8-7)
- Use for the proper amount of **TIME** (DRT)
- 1\textsuperscript{st} Remove **organic debris** with detergents and rinse well (rinsing is important) There is no ALL-IN-ONE-USE Disinfectant!
- Change the “general” disinfectant every 5 yrs.
- Select your **AGENT** according to your “PROBLEM” microbe! & LOG GROWTH!
EVALUATION of CHEMICAL AGENTS:

- **PHENOL COEFFICIENT** – a standard disinfectant first introduced by Lister
  
  - Uses a scale for each standard microbe individually where 1 = Phenol and 2 = twice the effectiveness of Phenol while 0.5 = 50% effective as Phenol
  
  - The standard microbes used are *Salmonella typhi* & *Staphylococcus aureus*
EVALUATION of CHEMICAL AGENTS:

FILTER PAPER DISC METHOD – this is a process done similar to the Kirby-Bauer Antibiotic Disc. A bacterial lawn is placed on an agar plate and discs soaked (but dry) with the chemical agent are placed on the agar and incubated at the opt. temp. for the microbe being tested. The resulting Zone of Inhibition indicates the relative “killing power” of the chemicals tested.
EVALUATION of CHEMICAL AGENTS:

USE DILUTION TEST – Dilutions of the test microbe are coating on metal cylinders and allowed to dry. The cylinder is dipped into several dilutions of the test chemical for 10 min. removed and rinsed with water. The cylinder is then placed into a nutrient broth solution at Opt. Temp. overnight. into a nutrient broth solution. The greatest dilution with NO CLOUDINESS is considered the best...
MECHANISMS OF KILLING

Denuration of Proteins: Hydrogen & Disulfide bonds altered

- BACTERIOSTATIC if temporary
- BACTERIOCIDAL if permanent

- HYDROLYSIS (Acids, Alkalis)
- OXIDATION (Hydrogen peroxide, Potassium permanganate, & Halogens)

- Attachment of atoms or chemical groups (Heavy metals, Alkylating agents, Halogens, and Alcohols)
MECHANISMS OF KILLING

ACTIONS ON MEMBRANES: since membranes contain proteins the previous also applies to the protein portion of the membrane as do chemicals that dissolve the lipid portion of the membrane PLUS..

- **SUFACTANTS** – soluble compounds that reduce surface tension (alcohol, detergents, & Quats.)
- **WETTING AGENTS** - detergent solutions used to penetrate membranes as “carriers” of antimicrobial agents and get rid of lipids and allow penetration
- **PHENOLS** - which are alcohols that dissolve lipids and at the same time denature proteins
SPECIAL COMMENTS 1:

- **Soaps** only emulsify oils and mechanically remove microbes except that true soaps have alkali and sodium and kill many species of Strep, Micrococcus, Neisseria, and Influenza viruses.

- **Bacteriocidal Soaps** have little increased effect.

- **Surgical scrubs** are effective but by Rx only.

- **Alcohols** need TIME to work are bacteriostatic usually & are only effective in 70-85% concentrations in water.

- **Detergents** are cationic (+) & used in sanitization or anionic (-) as in clothing detergents (not as effective as bacteria cell walls are negative in charge).

- **Quats** function poorly in the presence of soap, porous materials, Ca or Mg ions & encourage Pseudomonas~
5% bleach or Hyperchlorous acid (Cl + water) is a good agent if you remember that it destroys color and pits metal & is inactivated by organic debris.

Clothing Dryers are effective against many microbes.

Organic acids (like benzoic & ascorbic acid – Vit. C) is used as a preservative to retard microbial growth in soda pop, ketchup, margarine - works by lowering the pH.

Lactic acid and proprionic acids retard mold.

Tincture is a heavy metal (Mercury) dissolved in alcohol.
SPECIAL COMMENTS 3:

- PHENOLICS – organic matter does NOT affect (sometimes combined with Halogens for + effectiveness – hexachlorophene, Hibiclens)
  - Lysol, Amphyl, Cresol (carcinogenic)

- Heavy metals – selenium, mercury, copper, and silver (coins?)
  - Thimersol – an organic mercury used in eyewashes, skin, an instrument cleaning & vaccines
  - Selenium sulfide - kills fungi + spores in shampoos

- OXIDIZING AGENTS – hydrogen peroxide (enzymes inactivate this quickly) & potassium permanganate
SPECIAL COMMENTS 4:

- **HALOGENS** – Cl, Br, I etc.
  - Iodophores – iodine plus a slow releasing organic compound
  - Bromine (as methyl bromide) fumigant and hot tubs b/c of the “smell” of Cl

- **ALKYLATING AGENTS** – ‘aldehydes (carcinogenic) & ethylene dioxide (carcinogenic, irritant, penetrating, explosive) disrupt proteins + nucleic acids + kills endospores

- **DYES** – acridine (mutagen) methylene blue, crystal violet,

- **PLANT OILS** – Thymol, Eugenol (cloves)

- Sulfites(*wine, meat), Nitrites, sulfur dioxide (dried fruit, cereals), sodium diacetate (prevents mold in bread)…
Terms Related to Microbial Control (Chemical):

- **Thermal Death Point** – the temperature that kills 100% in a 24 hr culture at pH 7 in 10 min. (specific to species)

- **Thermal Death Time** – the time required to kill 100% at a certain temperature

- **Decimal Reduction Value** – (DRT or D value) found on the label with Opt. Temp. and Concentrations Recommendations – the time needed to kill 90% of the microbes in a given population at a specified temperature
PHYSICAL CONTROL of Microbes

- **DRY HEAT** (Flaming, Baking/Stoves)
- **MOIST HEAT** (Steam; Autoclave & Canning)
- **PASTEURIZATION**
  - Low Temperature
  - **UHT** (74.1 C to 140 C in 5 sec. = STERILE)
- **REFRIGERATION, FREEZING, FREEZE-DRYING**
- **RADIATION** (Ionizing, UV, Strong Light, Microwave, Sonic & Ultrasonic waves)
- **FILTRATION** (0.02 micron filter)
- **OSMOTIC PRESSURE**
PHYSICAL CONTROL of Microbes

- **DRYING & SMOKING** – used for centuries to preserve meat as absence of water damages enzymes; Smoking does not kill microbes; laundry drying kills many microbes...

- **DRY HEAT** (Flaming, OVEN/Baking/Stoves)
  - Damages by oxidation
  - Ovens used for glassware and metals, oils and powders... requires **MUCH TIME** (121 C for 16 hrs)!
  - Flaming loops etc. – watch for floating ashes and flying aerosols
PHYSICAL CONTROL of Microbes

- **MOIST HEAT** (Steam; Autoclave & Canning)
  - Very IMPORTANT b/c of penetrating ability
  - Boiling only destroys vegetative cells NOT endospores but the addition of 2% sodium bicarbonate can increase its effectiveness
  - Canning is similar to Autoclaving at 15 lbs/sq inch at 121 C for 15-20 min. kills all known microbes and endospores
    - Proper wrapping and proper placement in the autoclave
    - Autoclave Tape (?)
    - Test strips to test monthly (B. stereothermophilus)
PHYSICAL CONTROL of Microbes

PASTEURIZATION (Milk, Beer, Juices, etc.)

- Low Temperature (most US milk, spoils in 10 days must be continuously refrigerated) Flash or Hold Method; F=71.6°C for 15 sec. or 62.9°C for 30 min. NOTE: DOES NOT STERILIZE! Problems with Listeria & Coxiella bacteria!

UHT (STERILE – 74°C to 140°C and back to 74°C in 5 seconds plus cooling; must be refrigerated AFTER opening only) Canned and Paperboard boxed milk...
PHYSICAL CONTROL of Microbes

- REFRIGERATION, FREEZING, FREEZE-DRYING:
  - DOES NOT kill microbes!
  - It is “bacteriostatic” in that it SLOWS or STOPS bacterial replication
  - SLOWS or STOPS all enzymes
  - Normal refrigeration results in “DRYING”
  - DO NOT Freeze, Thaw and re-freeze!
  - Freeze-drying (lyophilization) is used to PRESERVE bacterial cultures...
PHYSICAL CONTROL of Microbes

- **RADIATION:**
  - **UV** – y b/w 40-390 (200 y usually) damages DNA and proteins as well as nucleic acid damage; inactivates viruses well, but some bacterial repair enzymes defeat the killing effect; **DOES NOT PENETRATE** and requires a lot of **TIME**!
  - **Ionizing** - Xrays (0.1-40 nm y) and gamma rays (0.3-0.4 millirads) are used in “mass” sterilization of goods such as rubber gloves, plastic items such as syringes and baby bottles. Also pharmaceuticals, meats, fruits, and vegetables are now sterilized in this manner. Gamma radiation destroys nucleic acids, produces peroxides, and releases electrons and leave **NOTHING BEHIND** – **IT IS COMPLETELY SAFE** and is relatively cheap for bulk sterilization.
PHYSICAL CONTROL of Microbes

- **RADIATION:**
  - **Strong Visible Light** – (violet to red; 400-700 nm) has a bactericidal effect by oxidizing light sensitive molecules like riboflavin and porphyrins. Eosin and Methylene Blue dyes are activated in strong light to damage proteins & nucleic acids and have been used to rid materials of bacteria and some viruses.
  
  - **Microwaves** – long wavelength radiation (1 mm – 1 m) causes water turns to steam; usually does not sterilize thick items; specialized microwave ovens are now used in certain situations & are effective.
PHYSICAL CONTROL of Microbes

- RADIATION:
  - Sonic (SONICATION) & Ultrasonic waves (15,000 cycles; cause CAVITATION) – in the audible range can destroy bacteria if of sufficient intensity; STERILIZES BUT NOT PRACTICAL for use; used to produce cell fragments for studys
  - FILTRATION – DOES NOT STERILIZE! It is used in live virus vaccine production; liquids require VERY small pore size (0.20 um) but do NOT prevent contamination with Mycoplasmas & Ureaplasmas (bacteria without a cell wall). HEPA filters used to filter but not sterilize air... Can be used to separate viruses for production...
PHYSICAL CONTROL of Microbes

- **OSMOTIC PRESSURE** – High concentrations of salt or sugar cause **PLASMOLYSIS** – loss of water, due to the effect of osmosis. have been used for centuries to preserve meats, syrups and fruit juices (jam and jelly) from contamination. Does not prevent **Halophilic** bacteria in pickle manufacturing or **MOLD** in cheese and jellies from growing.