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# **Controlling Microbial Growth**

Lecture 10


## **Learning Outcomes**

- The language of Sterilization
- Molecular Targets for disinfectants
- Non-chemical methods of sanitization
- Chemical disinfectants

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# **Killing Microbes**

- Disinfection
  - Killing vegetative cells
  - Non-living surfaces
- Sanitization
  - Lower microbial counts
- cutlery
- Degerming
  - Swabbing skin before shot
- Antisepsis
  - Disinfection of living tissue
- Sterilization
  - Surgical instruments

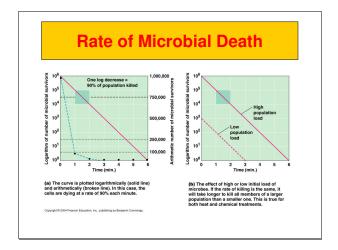



# Killing Terminology

- · ~ide
- Death to microbes
- ~stat or stasis
  - Stop population growth without killing living cells
- Sepsis Greek putrid




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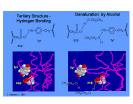


# Targets of Microbial Control Agents Plasma Membrane Alteration of permeability Proteins Denaturation Enzyme inhibition Nucleic Acids Inability to replicate Oxidizing SH2 group of cystine part of many enzymes Oxidizing agents (Chlorox) denature these enzymes

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## **Alcohol**

- · 95%
- Coagulation of outside surface proteins
- 70%
- · Infiltrates cell
- Denaturation by disruption of H bonds






#### Sterilization

- Commercial canning
- · Thermal death Point
  - Lowest temp all microbes killed in 10 minutes

#### · Thermal Death Time

- Minimum time all bacteria killed at a given temp
- Decimal Reduction Time
  - Minutes to kill 90% bacteria at a given temp
- 12D botulinum endospores
  - Not sterile

## Heat




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## **Moist Heat**

- · Kills by denaturization
- · Boiling (10 min)
  - Kills vegetative bacterial cells
  - Almost all viruses, fungi and spores
  - Altitude
- Endospores can resist 20 hours of boiling



# Higher pressure = Higher heat 15 psi = 121 °C Kill all organisms + endospores 15 mins Cannot be used with heat labile materials Some plastics, rubber Comparison of the comparison of the

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## **Pasteurization**

- Originally to preserve wine and beer
- Eliminate most pathogens but does NOT achieve sterilization
- Lower microbial count
- 72 °C 15 seconds
  - refrigeration
- · Ultra Heat Treatment
- · milk is sterilized
  - $74^{\circ}C \rightarrow 140^{\circ}C \rightarrow 74^{\circ}C$  5 seconds
  - Store @ room temp




# **Dry Heat**

- Kills by oxidation
- Flaming
- Incineration
  - Hospital wastes
- Hot air sterilization
- Oven temp >170 °C 2 hours



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## **Filtration**

- Sterilize heat sensitive media
- Air filters
- · HEPA filters
  - To 0.3 um
  - Operating Room
  - Burns Units
- Membrane filters
  - 0.45-0.22 um bacteria
  - 0.01 um viruses






## Refrigeration

- Refrigeration 0-7 °C
  - Bacteriostatic
  - Most pathogens do not grow
- Listeriosis
  - Listeria monocytogenes
  - Can grow at 4 °C
- Freezing





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# High Pressure

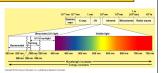
- · Alters protein structure
- · Inactivation of bacterial vegetative cells
- · Endospores resistant
- Fruit juices
- Does not alter flavour, colour, nutritive value



## **Ionizing Radiation**

- < 1nm
- Gamma
- X-rayHigh energy electron beams

- Ionization of water → hydroxyl radicals OH
  DNA alteration
  High energy electrons
  Preservation of fruit and vegetables
- Sterilize pharmaceuticals
  Disposable syringes, gloves etc.



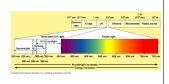


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#### - > 1nm

- UV radiation 260nm

- Thymine dimers
  UV light sterilization
  Sterilization of vaccines
  UV Lamps
- Kill airborne microbes
- Does NOT penetrate glass OR plastics
- Sunlight weakly antimicrobial
- Formation of singlet oxygen in cytoplasm
- Microwaves not antimicrobial
- Friction boils water creating steam



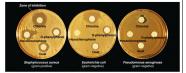
**Non-Ionizing Radiation** 



#### Disk Diffusion Method

- · Filter paper soaked in disinfectant
- Placed on plate inoculated with target organism
- Larger zone of Inhibition = greater susceptibility to disinfectant

## Evaluating a **Disinfectant**



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## **Phenols, Phenolics and Bisphenols**

- · Lister's Carbolic Acid
- Phenolics
  - Less irritating
  - Damages lipid-rich plasma membranes
  - Mycoplasma
  - Active in presence of organic compounds
  - Lysol
- Bisphenols
  - 2 phenol rings
  - Hexachlorophene
  - Effective: Gram +ve Staph + Strep
  - Triclosan





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#### · Chlorhexidine

- Broad spectrum antiseptic
  - Surgical prep
- Disrupts plasma membrane
- Effective against most vegetative bacteria
- Not effective:
  - Mycobacteria
  - Endospores
  - Protozoan cysts

## **Biguanides**






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lodine

- Antiseptic
- Effective against
  - All kinds of bacteria
  - Many endospores
  - Various fungi
  - Some viruses
- · Damages: Enzymes?
- Tincture
- · Iodophor
  - Betadine
  - Isodine



- Cl₂ gas Dissolves in H₂O→ Hypochlorus acid
- · Oxidizing agent
  - Affects proteins with sulfhydryl (SH) groups
  - Nucleic acids
  - Swimming pools
- Drinking water
- Sodium hypochlorite NAOCI
  - Clorox
  - Chloramines
  - Sanitize glassware





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# **Ethylene Oxide**

- Gas
- Used to sterilize objects too large to autoclave
- Mattresses
- · Space shuttles



#### · Inactivate proteins

- Formaldehyde
- Glutaraldehyde
  - Less irritating
  - More effective
- Disinfect hospital instruments
- [2%] batericidal, tuberculocidal, viricidal in 10 mins
- · Sporicidal 3-10 hours
- OPA orthophthalaldehyde

## **Aldehydes**






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- Biocides
- Silver
  - Silver impregnated dressings
    - Silver sulfadiazine
- Mercury chloride
  - Bacteriostatic
  - toxic
- Copper sulfate
  - Algicide
  - Reservoirs
  - Swimming pools

## **Heavy Metal**



## **Quaternary Ammonium** Compounds

- Strongly bactericidal v Gram + ve
   Fungicidal
   Amoebacidal
   Envelope viruses
- Not effective
  - Endospores
  - Mycobacteria
  - Pseudomonas actively grows in them
- Zephiran
- Cepacol
- · Antrimicrobial
- · Colorless, odorless, tasteless





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- · Retard food spoilage
- Sodium nitrite
   Bacon
- · 50<sub>2</sub>
- wine
- · Sodium benzoate
- Acidic foods
  - juices + sodas
- Sorbic acid
- Cheeses + winesCalcium propionate
- Inhibits molds
  - bread

# Chemical Food Preservatives




### · Oxidizing agents

- Ozone
  - Drinking water
- Hydrogen Peroxide
  - Sporicidal
  - Sterilization of contact lenses
- · Benzoyl Peroxide
- · Peracetic acid
- Legionella








### **Microbial** Characteristics

- Many biocides more effective vs Gram +ve
- · Gram -ve cell wall
- porins
- Biocide resistance
- Pseudomonas
- · Burkholderia
- Mycobacteria M.tuberculosis