CONTROL OF MICROBIAL GROWTH
Microbial Control

- Ignatz Semmelweis (1816-1865)
- In 1847, Dr. Ignaz Semmelweis's close friend, Jakob Kolletschka, cuts his finger while he's doing an autopsy
- Kolletschka soon dies of symptoms like those of puerperal fever leading Ignaz Semmelweis to pioneer antiseptic policy
  - Chlorinated lime
Joseph Lister

- Joseph Lister (1827-1912)
  - Joseph Lister had been convinced of the importance of scrupulous cleanliness and the usefulness of deodorants in the operating room; and when, through Pasteur's researches, he realized that the formation of pus was due to bacteria, he proceeded to develop his antiseptic surgical method.

- Carbolic acid (phenol)
Lister’s Carbolic acid sprayer
Joseph Lister

- Lister worked at a time of increased hospital sanitation and cleanliness. The filth and disease of earlier hospitals had been replaced with practices of disinfection, such as whitewashing walls and beds with nitrous oxide.
- General cleanliness through washing with soap and water and keeping dressings clean and fresh was also an emerging medical practice.
TERMS RELATED TO DESTRUCTION OF ORGANISMS
Control of Microbial Growth

- **Sterilization** – killing of all microbial life
  - (commercial sterilization – only hot enough to kill *Clostridium botulinium* canned food)
- **Disinfection** – destruction of vegetative (non-spore forming) pathogens mostly chemically – disinfectant
- **Antisepsis** – treatment of living tissue to kill pathogens - antiseptic
Control of Microbial Growth

• Degerming – removal of most microbes by swabbing with alcohol
• Sanitization – removal of most microbes from surfaces (dishes, glasses, etc.)
• Biocide – kills microbes
• Bacteriostatic – inhibits microbial growth
STERILIZATION (cont.)

- Moist heat, 121°C for 15 min
- Dry heat, 170°C for 120 min
STERILIZATION (cont.)

– Ionizing radiation
– Chemical (ethylene oxide)
DISINFECTION

• The process of destroying microorganisms

• Usually liquid chemicals
  – Some organisms may survive disinfection (Legionella pneumophila)
ANTISEPSIS
(anti-putrefaction)

• Disinfection of skin or tissues
  – Usually wounds
  – Surgery
CIDET = TO KILL

Germicide
Bactericide
Sporicide
Fungicide
Virucide
TERMS RELATED TO SUPRESSION OF ORGANISMS
BACTERIOSTASIS

• Multiplication of organisms is inhibited
• May continue when the bacteriostatic factor is removed
ASEPSIS (A = without)

- Absence of microorganisms from an object or area
Sanitization

• The reduction of organisms on inanimate objects
  – Eating utensils
  – Dairy equipment, etc.
CONDITIONS INFLUENCING MICROBIAL CONTROL
TEMPERATURE

- low, inhibits growth
- high, promotes disinfectant activity
TYPE OF MICROORGANISM

• Gram positive generally more sensitive to disinfection

• *Pseudomonas* spp. resistant to disinfection
Actively growing organisms are more susceptible to disinfection
Environment

- Organic matter protects against chemical or physical inactivation
MODE OF ACTION

• Alteration of lipids and proteins of cytoplasmic membrane
  – Quaternary ammonium compounds
MODE OF ACTION
(cont.)

• Alteration of proteins and nucleic acids
  – Enzymes
  – Reproduction
  – Synthesis of proteins
RATE OF MICROBIAL DEATH

death does not occur simultaneously
Bacterial Death Rate

Number of bacteria

Time

1 \times 10^4

1 \times 10^3

1 \times 10^2

1 \times 10^1
1,000,000
E. coli

1,000
E. coli
PHYSICAL METHODS OF DISINFECTION

- Heat
- Filtration
- Freezing
- Desiccation
- Osmotic pressure
- Radiation
Physical Methods

• Radiation
  – Ionizing Radiation (<1 nm, high energy) gamma rays, X-rays, high energy electron beam
    • Creation of hydroxyl radicals from water, DNA damage
    • Certain foods, pharmaceuticals, surgical supplies, plastic syringes, etc.
  – Non-Ionizing Radiation (>1nm, lower energy) UV light, DNA damage (260 nm Thymine dimers)
    – (microwaves) – inadequate
Physical Methods

• Desiccation
  – Very variable effect on microbes, many can survive extended times (months), some endospore for centuries, others die in minutes
  – Great significance for hospital & nursing home microbiology, bedding, dust, clothing contaminated with mucus, urine, feces or pus

• Osmotic Pressure
  – High salt or sugar content (hypertonic conditions)
Chemical Methods - Disinfectants

- **Gas sterilizers**
  - E.g.: ethylene oxide
  - Denatures proteins via alkylation of –SH, –COOH, -OH groups
  - Highly penetrating
  - Used in closed chambers, industrial sterilization of plastic-wrapped syringes, needles, tubing etc.
  - Hospitals (large chambers to treat entire mattresses)
HEAT

• Moist heat
• Dry heat
• Pasteurization
MOIST HEAT

• Boiling (100° C)
  – Kills nonspore-forming bacteria, viruses, mold and fungi

• Pressurized (121° C)
  – All organisms (but prions)
Autoclave
DRY HEAT

• Flaming
• Hot air sterilization, 170º C, 2 hours
Physical Methods

• Heat – destruction of proteins
  – **Autoclave** – 121°C 1 atm pressure for 15 min will kill all organisms and their endospores
  – **Pasteurization** – milk, yogurt, beer, etc. brief heating kills most spoiling microbes without altering taste (milk 72°C 30 s)
  – **UHT** – ultra high temperature treatment (coffee creamer, etc. need no refrigeration (140 °C ca. 2 s)
• **Dry Heat** – direct heat by flame or hot air (170°C) for 2 h
Sterilizing Oven

This Class 100 Sterilization Oven is used for the depyrogenation of glass, teflon, and other heat resistant materials.
Physical Methods

• Sterile Filtration
  – For heat sensitive solutions/liquids
  – Cellulose esters or plastic polymers (pore size from 0.45 – 0.01 µm)
  – High Efficiency Particulate Air Filters (HEPA) filters (pore size 0.3µm)

• Cooling/Freezing
  – Refrigeration – (0-7°C) only bacteriostatic
  – Slow freezing kills many pathogens and parasites
LIQUID FILTRATION

- Culture media
- Laboratory reagents
- Pharmaceutical products
GAS FILTRATION

• High-Efficiency Particulate Air (HEPA) filters
  – Laminar flow hoods
  – Hospital rooms
Laminar flow hood

Used in cell culture
Semiconductor industry to produce wafers for cell phones
Chemical Methods - 
*Disinfectants*

- **Heavy Metals**
  - Ag, Hg, Cu
  - Reaction of −SH groups on proteins with minute amounts of metal ions
  - Burn victims, dressings with AgNO$_3$ and a sulfonamide (antibiotic)
  - (Hg in paints prevention of mildew)
  - (CuSO$_4$) potent against algae in swimming pools, fish tanks, etc.
Heavy Metals

- Silver (Ag) is an antimicrobial agent
- Ag impregnated dressings for burned victims
- Ag is also incorporated into indwelling catheters
Copper

• $\text{CuSO}_4$ used to destroy green algae in swimming pools and fish tanks
Zinc

- Cu + Zn treated shingles are available to create anti-fungal roofs
- ZnCl₂ is a common ingredient in mouthwashes
Chemical Food Preservatives

- $\text{SO}_2$ (wine)
- Na-benzoate, Ca-propionate, sorbic acid (cheese, soft drinks, breads)
- $\text{NaNO}_3$ or $\text{NaNO}_2$ (ham, bacon, hot dogs)
  - Very effective against *Clostridium botulinum*
Chemical Food Preservative

• Nabenzoate in coke/cheese prevents growth of molds
• Foods w/ low pH tend to be susceptible to mold
Nitrates/Nitrites

- NaNO$_3$/NO$_2$ are added to meat products (ham, bacon, hot dogs etc.)
- Salts prevent growth of some types of bacteria that are responsible for meat spoilage
Nitrates in food

- Salts prevent bacterial growth via the drying effect (most bacteria require substantial amounts of moisture to live/grow)
- A preference developed for certain salts that produced a pink color and special flavor in meat
- Near the turn of the century it was determined that sodium nitrate was responsible for this special and flavor
- Nitrate changes to nitrite by bacterial action during processing and storage
Nitrates in food

Nitrite in meat greatly delays the development of botulinal toxin (botulism)

Sugar is added to cured meats as well to reduce the harshness of salts

Sodium nitrite (NaNO₂), rather than sodium nitrate (NaNO₃), is commonly used for curing

NO₂ is converted to Nitric oxide

Nitric oxide combines with myoglobin (responsible for the natural red color of uncured meat)
Nitrates in meat products

• \( \text{NO}_3 \) is converted to \( \text{NO}_2 \)
• \( \text{NO}_2 \) provides meat the red color
• Nitrates combine with amino acids
Nitrosamines

• prevents botulism

• is a carcinogenic product

• however body produces plenty of nitrosamines from other sources
Disk Diffusion Method
Kirby-Bauer Method

• Disk of filter paper soaked in disinfectant or antibiotic

• Place on agar previously inoculated with test organism
Disk Diffusion Method

- Incubation
- Measure inhibition zone
The earliest recorded example of chemical disinfection is the use of copper or silver vessels, instead of pottery ones, to store drinking water to prevent it becoming foul.

This innovation was introduced about 450 BC by the Persians.

Both copper and silver have significant antimicrobial activity, although neither is much used for disinfection purposes today because of their toxicity.
Disinfectants

Other ancient disinfectants, used mainly for topical treatment of wounds were wine, vinegar and honey, while wine and honey now tend to be used internally, vinegar, or rather its active ingredient, dilute acetic acid, has been revived as a wound dressing where antibiotic resistant *Pseudomonas* bacteria are a problem.
Disinfectants

Mercuric chloride was introduced as a wound dressing in the Middle Ages by the Arabs, but it was not until the 18th and 19th centuries that great strides forward in chemical disinfection were made with the introduction of a range of chemicals such as copper sulphate (1767), bleaching powder (1798), creosote (literally, 'flesh-saviour' from the Greek; 1836), iodine (1839), chlorine water (1843) and phenol (1860)

Today, some of these are still used for some disinfection purposes, and there is a large array of more modern chemicals
TYPES OF DISINFECTANTS

• Phenol and phenolics
• Chlorhexidine
• Halogens
• Alcohols
• Heavy metals
TYPES OF DISINFECTANTS

- Surfactants
- Quaternary ammonium compounds (quats)
- Organic acids
- Aldehydes

On a box of Willert Bowl Fresh:
Safe to use around pets and children, although it is not recommended that either be permitted to drink from toilet.
Chemical Methods - Disinfectants

- **Alcohols**
  - 70% ethanol, or isopropanol ("rubbing alcohol")
  - Mode of action: Protein denaturation & coagulation therefore \(\rightarrow\) membrane disruption
  - Advantage of evaporation after disinfection
  - Poor effect on wounds! Superficial coagulation of proteins \(\rightarrow\) secludes deeper layers of microbes
Ethanol (ethyl alcohol) and Isopropanol

most frequently used alcohols

Ethanol: CH$_3$CH$_2$—OH  Isopropanol: CH$_3$—CH—OH

CH$_3$
Effect Ethanol

Alcohols kill vegetative forms of bacteria (including TB) and fungi, but have no action on spores or viruses.

Their effect depend on concentration and type of alcohol.

The following three solutions have similar effect: Ethanol 70%, isopropanol 60% and n-propanol 40%. 70-80% alcohol inactivates HIV and Hepatitis B in 2-10 minutes.
How does it work?

Alcohols precipitate proteins and solubilize lipids present in cell membranes.

It has a rapid action.

Contact time should preferably be 10-30 minutes.
Phenols

These compounds, derived from coal tar, were first used as wound dressings, but today have a wide use as general disinfectants.

Examples are 'Lysol' (cresol and soap solution) and 'Stericol' (xylenol-rich cresylic acid and soap solution), both of which are active against viruses and bacteria but less active against bacterial spores.
Phenolics and QUATS

Since Lister showed in 1867 that phenol (carbolic acid) would kill microorganisms, many chemicals have been tested for this purpose.

Phenols are considered to be low-intermediate level disinfectants.

Phenol no longer used as a disinfectant because of its toxicity to tissues.

Many non-pathogens attack organic matter, producing chemicals which may be highly odorous, corrosive or staining.
Phenols and QUATS

hard surface disinfection chemicals

1. Quaternary ammonium compounds
2. Phenolic compounds
Chemical Methods - *Disinfectants*

- Efficacy evaluated via standard test methods (standard organisms)
- **Phenol & Phenolics**
  - Mode of action: Damage lipid cell membranes
  - Quite effective in the presence of organic materials (pus, saliva, feces)
  - E.g. Lysol® (O-phenylphenol)
  - Very good surface disinfectants
QUATS and PHENOLICS

• Cleaning non-critical environmental surfaces

• Products used for this purpose generally contain low-level disinfectants such as quaternary ammonium compounds (quats) or phenolics

• Effective against easier to kill vegetative bacteria

• Excess soil is first removed

Minimum of 10 minutes of contact time

In most situations neither condition is met to make the disinfection successful

Such chemicals may also have the potential to make bacteria more resistant to antibiotics
Quats/Phenolics

• Quats and phenolics have negative effects on humans
• Are considered to be hazardous by OSHA criteria
• Some products registered as disinfectant cleaners may claim to be non-hazardous because they haven’t been tested yet
• They should not be considered free of toxic health effects
PHENOL

- Carbolic acid
- Skin irritant
- Throat sprays (septosol)
- 100 mL of SEPTOSOL Throat Spray contains 0.5 g phenol with sodium benzoate 0.30% m/v as preservative---temporary relief of throat pain
PHENOLICS (cont.)

• Active and stable in the presence of organic mater
  – Saliva, feces and pus
  – Cresols (o-phenylphenol)
  – Long lasting, very good surface disinfectants (Lysol)
PHENOLICS

• Phenol derivatives
  – Less of an irritant, and more effective than phenol

• Mode of action
  – Damages plasma membrane
  – Enzyme inactivation
  – Protein denaturation
PHENOLICS (cont.)

• *Hexachlorophene*
  – Against Gram positives
  – Currently used in hospitals (scrubbing)
  – Neurotoxic

• infants
SOAPS
SOAPS

• mechanical removal of microbes through scrubbing

• soap breaks oily film on skin (emulsification); little value as an antiseptic

• detergents - more effective against gram positive than gram negative
  -- nonionic detergents have no germicidal activity
  -- anionic (acid) sanitizes, food and dairy industry
  -- cationic (positive)-antiseptic for skin, instruments, utensils
Surfactants
Chemical Methods - Disinfectants

- **Surfactants** (surface active agents)
  - “Soaps” *old fashioned* Na/K salts of fatty acids (Na/K + animal fat = glycerol)
  - Detergents modern surface active agents
  - Emulsification of skin oils & dead cells → cleansing of skin and other surfaces
  - Some destruction of cell membranes (at high concentrations, cell lysis)
Surfactants

Surface active agents (surfactants).

4 types, cationic, anionic, non-ionic & amphoteric agents

the anionic & non-ionic compounds have only very weak antimicrobial activity

They are active against bacteria and enveloped viruses (such as feline herpesvirus) but not non-enveloped viruses (such as feline calicivirus)
Surfactants

• main use is in wound disinfection and as preservatives in pharmaceutical preparations, eg. eye drops.

• amphoteric compounds (ie. possess both anionic and cationic characters) are the most active disinfectants of the 4 types of surfactants, and are less affected by organic matter.
Surfactants

Surfactants are non-ionic, usually alcohol ethoxylates which are more efficient at penetrating skin than many other surfactants.

Surfactants enhance the toxicity of other toxic chemicals and concerns have been raised about low-level exposure to toxic chemicals.
TYPES OF DISINFECTANTS (cont.)

• Gaseous chemosterilizers (ethylene oxide—effective all forms of life incl. endospores)

• Oxidizing agents (Chlorine)
Chemical Methods - *Disinfectants*

**Oxidizing agents**

- O$_3$ (Ozone) drinking water
- H$_2$O$_2$
  - effective on inanimate objects, but ineffective on open wounds due to destruction by Catalase
  
  $\quad \text{\textit{\(2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2\)}\text{\textit{}}}$
  - Contact lens sterilization
- Benzoyl peroxide
  - Irrigation of wounds infected by anaerobic bacteria, acne treatment (anaerobic bacteria in hair follicle)
Chemical Methods - Disinfectants

- Oxidizing agents
  - Peroxyacetic acid
    - Most effective sporicide, sterilization of food processing and medical equipment
    - Leaves no residue (disintegrates into $O_2$ and acetic acid)
Chemical Methods - *Disinfectants*

- **Bisphenols**
  - E.g. hexachlorophene
  - Mechanism ?
  - Used in OR, hospitals, nurseries
  - Very effective against gram+ staphylococci causing skin infections in newborns

- **Biguanides**
  - E.g. chlorhexidine
  - Damage lipid cell membranes
  - Surgical hand scrub, preoperative skin disinfection (combined with soaps & alcohol)
CLORHEXIDINE

• A biguanide (non-phenolic)
• Skin and mucous membranes, low toxicity
• Surgery
  – Scrubbing and skin preparation
Chlorhexidine

**Effect:**

- Chlorhexidine is efficient against both gram+ and gram- bacteria (*Pseudomonas, Proteus and Providencia* might be resistant).
- No effect is seen on TB, spores and viruses.
- The bactericidal effect is enhanced by alcohol.
Organic Acids
Organic Acids - various organic acids and their salts are common antimicrobials in foods

- preservatives to control mold growth

- sorbic acid (Ca, Na, K) used in cheeses, baked goods, soft drinks, fruit juices, jams, jellies

- benzoates (sodium benzoate, methy-p-hydroxybenzoate [methylparaben]) fruit juices, jam, jellies, soft drinks, salad dressings, margarine, and many pharmaceutical products

- boric acid used in eye washes

- calcium propionate-prevents mold growth in bread
HALOGENS

- Iodine
- Chlorine
Chemical Methods - *Disinfectants*

- **Halogens**
  - Iodine $I_2$
  - Halogenation of microbial proteins
  - Iodine tincture (solution in diluted alcohols)
  - Iodophores – organic molecules slowly releasing $I_2$, less staining than straight $I_2$ preparations (e.g. Betadine®, Isodine®)
  - Extremely effective, skin disinfection, wound treatment ($I_2$ tablets water treatment)
IODINE \((I_2)\)

- Wide spectrum
  - Antiseptic
    - Bacteria, viruses, many endospores and fungi
- Combines with tyrosine and oxidizes -SH groups
  - Alters proteins
IODINE (cont.)

- Skin irritant
- Tincture
  - $\text{I}_2 + \text{alcohol}$
- Iodophor (Betadine and Isodine)
  - $\text{I}_2 + \text{organic molecule}$
  - Less irritant
  - *Pseudomonas* resistant
Chemical Methods - *Disinfectants*

• **Halogens**
  – Chlorine $\text{Cl}_2$ (gas)
  – Formation of $\text{ClO}^-$ in water ("bleach")
  – Strong oxidizing activity destroys microbial enzymes
  – Drinking water, swimming pools, sewage treatment
CHLORINE (Cl\textsubscript{2})

- 1846 Semmelweiss
  - Puerperal fever
- Since 1908 main water disinfectant in the USA
CHLORINE (Cl₂) (cont.)

Cl₂ + H₂O ↔ H⁺ + HOCl
- Hypochlorous acid

HOCl ↔ H⁺ + OCl⁻
- Hypochlorite ion
HOCl, OCl⁻ and pH
Disinfection %

Cl₂ disinfection and pH

pH 4

pH 10
HYPOCHLORUS ACID VS. HYPOCHLORITE

• Hypochlorous acid has no charge (neutral) HOCl
  – Moves freely through membrane
HYPOCHLORUS ACID VS. HYPOCHLORITE (cont.)

- Hypochlorite ion negatively charged (HCl⁻)
- Cannot enter cell freely
CHLORINE ACTION

• Strong oxidizing agent
• Interferes with enzyme activity
CHLORINE LIMITATIONS IN WATER DISINFECTION

• Generates toxic trihalomethanes
  – Chloroform related chemicals
CHLORAMINES

- $\text{Cl}_2$ and ammonia
- Long lasting, but slow
- Effective with organic matter
ALCOHOLS

• Not strong disinfectants
• Kill bacteria, fungi and enveloped viruses
• Do not kill endospores, or naked viruses
ALCOHOLS (cont.)

- Ethanol
- Isopropanol
ALCOHOLS ACTION

- Dissolves lipids
- Denatures proteins
  - Needs water
  - More effective when diluted (usually 70%)
HEAVY METALS

• Silver
• Mercury
• Copper
OLIGODYNAMIC ACTION

• The ability of trace amounts of heavy metals to disinfect

• Heavy metals denature proteins by combining with -SH groups
SILVER

• *Silver nitrate*
  – Gonorrheal ophthalmia
  – Rarely used
MERCURY

• *Mercuric chloride*
  – Toxic and corrosive
  – Mildew in paints
COPPER

- Copper sulfate
- Algicide
  - reservoirs, swimming pools, fish tanks
- Mildew in paints
SURFACTANTS

• Soaps
• Detergents
• Mechanical removal of microorganisms
SURFACTANTS (cont.)

• Acid-anionic detergents
  – Sanitizer of dairy equipment
  – Nontoxic, noncorrosive and fast acting
QUATERNARY AMMONIUM COMPOUNDS (QUATS)

- Cationic detergents
- Strongly bactericidal
- Gram + are more susceptible than Gram -
QUATS (cont.)

• Do not kill naked viruses, endospores, or *Mycobacterium tuberculosis*

• *Pseudomonas* spp. can grow on quats

• Zephiran and Cepacol
ORGANIC ACIDS

• Food preservatives
  – Safe in foods
  – Mold control in foods and drinks
    • Sorbic acid
    • Benzoic acid

• Mold control in cosmetics
  – Parabens
ALDEHYDES

• Formaldehyde and glutaraldehyde
• Strong antimicrobials
ALDEHYDES (cont.)

- Sporicidal
  - Inactivate proteins
- Used for vaccine preparation
- Used for embalming
GASEOUS CHEMOSTERILIZERS

- Ethylene oxide
- Denatures proteins
- Sterilizes (4 to 18 hours)
- Disposable plasticware
OXIDIZING AGENTS

• Ozone
  – Water disinfection
• Hydrogen peroxide
  – Inanimate surfaces