5. Control and Sterilization of Microorganisms

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2022/2023

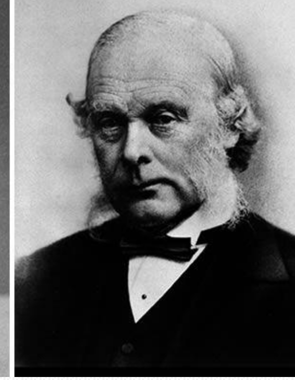
Lecture Outline

- Definitions
- Mechanisms
- Importance
- Conditions Influencing Antimicrobial Activity
- Physical Methods
- Chemical Agents

Definitions

- Sterilization: A treatment that kills or removes all living cells, including viruses and spores, from a substance or object
- Disinfection: A treatment that reduces the total number of microbes on an object or surface, but does not necessarily remove or kill all of the microbes
- Antiseptic: A mild disinfectant agent suitable for use on skin surfaces
- Sanitization The process whereby pathogenic organisms are reduced to safe levels on inanimate objects
- Biocide A chemical or physical agent, usually broad spectrum, that inactivates microorganisms

History

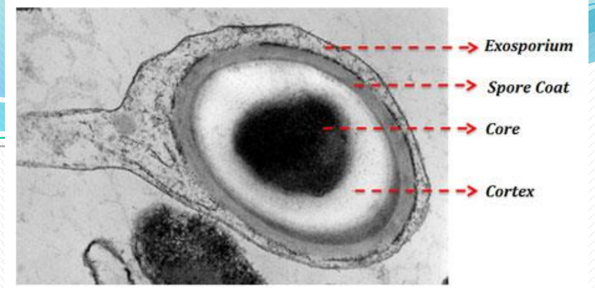


- British physician Joseph Lister (Father of antideptic)
  - "saved more lives by the introduction of his system than all the wars of the 19th century together had sacrificed."
  - Lister revolutionized surgery: introduced methods to prevent infection of wounds
- Until late 19th century, patients undergoing even minor surgeries were at great risk of developing fatal infections
- Modern hospitals use strict procedures to avoid microbial contamination

Importance

- Daily Life
- Water
- Food
- Pharmaceuticals
- Hospitals
- Microbiology Laboratories

Resistant Microorganism



Bacterial Endospore

- **Bacterial endospores**: most resistant, only extreme heat or chemical treatment destroys them
- **Protozoan cysts and oocysts**: resistant to disinfectants; excreted in feces; causes diarrheal disease if ingested
- **Mycobacterium species**: waxy cell walls makes resistant to many chemical treatments
- **Pseudomonas species**: resistant to and can actually grow in some disinfectants
- **Non-enveloped viruses**: lack lipid envelope; more resistant to disinfectants

Conditions Influencing Antimicrobial Activity

- Several critical factors play key roles in determining the effectiveness of an antimicrobial agent, including:
  - Population size
  - Types of organisms
  - Concentration of the antimicrobial agent
  - Duration of exposure
  - Temperature
  - pH
  - Organic matter
  - Biofilm formation

GENERAL MECHANISMS OF BIOCIDE ACTION

- Disruption of the Cell Membrane or Wall
- Protein Denaturation
- Disruption of Free Sulfhydryl Groups
- Damage to DNA
- Chemical Antagonism

Physical Methods

1. Moist Heat
2. Dry Heat
3. Low Temperatures
4. Filtration
5. Radiation

1. Moist Heat

- Mechanism of killing is a combinantion of protein/nucleic acid denaturation and membrane disruption
- Effectiveness Heavily dependent on type of cells present as well as environmental conditions (type of medium or substrate)
- Bacterial spores much more difficult to kill than vegetative cells

Methods of Moist Heat

- **Boiling at 100°C:** Effective against most vegetative cells; ineffective against spores; unsuitable for heat sensitive chemicals & many foods
- **Autoclaving/pressure canning:**
  - Temperatures above 100°C achieved by steam pressure
  - Most procedures use 121.1°C, achieved at approx. 15 psi pressure, with 15 - 30 min autoclave time
- **Pasteurization:**
  - Used to reduce microbial numbers in milk and other beverages while retaining flavor and food quality
  - Traditional treatment of milk, 63°C for 30 min
  - Flash pasteurization (high-temperature short term pasteurization); quick heating to about 72°C for 15 sec, then rapid cooling

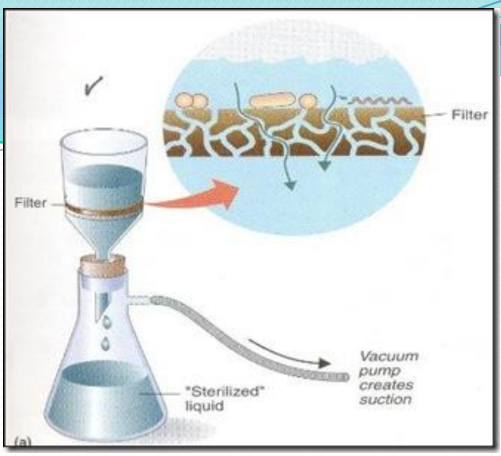
2. Dry Heat

- **Incineration**
  - Burner flames
  - Electric loop incinerators
  - Air incinerators
- **Oven sterilization**
  - Used for dry glassware & heat-resistant metal equipment
  - Typically 2 hr at 160°C is required to kill bacterial spores by dry heat: this does not include the time for the glass to reach the required temp (penetration time) nor does it include the cooling time

3. Low Temperatures

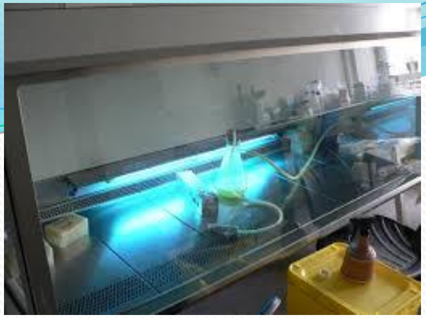
- **Refrigerator:**
  - around 4°C
  - inhibits growth of mesophiles or thermophiles; psychrophiles will grow
- **Freezer:**
  - "ordinary" freezer around -10 to -20°C
  - "ultracold" laboratory freezer typically -80°C
  - Generally inhibits all growth; many bacteria and other microbes may survive freezing temperatures

4. Filtration



- Used for physically removing microbes and dust particles from solutions and gasses; often used to sterilize heat-sensitive solutions or to provide a sterilized air flow
- **Depth filters**: Thick porous filtration material (e.g., cellulose), Larger pores, Electrical charges trap cells
- **Membrane filters**: Small pore size (0.2 μm) to remove bacteria, Thin, eg. Nitrocellulose, nylon, polyvinylidene difluoride
- **HEPA filters**: **H**igh **e**fficiency **p**articulate **a**ir filters used in laminar flow biological safety cabinets

5. Radiation



- Ultraviolet Radiation
  - DNA absorbs ultraviolet radiation at 260 nm wavelength
  - This causes damage to DNA in the form of thymine dimer mutations
  - Useful for continuous disinfection of work surfaces, e.g. in biological safety cabinets
- Ionizing Radiation
  - Gamma radiation produced by Cobalt-60 source
  - Powerful sterilizing agent; penetrates and damages both DNA and protein; effective against both vegetative cells and spores
  - Often used for sterilizing disposable plastic labware, e.g. petri dishes; as well as antibiotics, hormones, sutures, and other heat-sensitive materials

Chemical Agents

1. Phenolics
2. Alcohols
3. Halogens
4. Heavy metals
5. Quaternary Ammonium Compounds
6. Aldehydes

1. Phenolics

- Aromatic organic compounds with attached -OH
- Denature protein & disrupt membranes
- Commonly used as disinfectants (e.g. “Lysol”); are tuberculocidal, effective in presence of organic matter, remain on surfaces long after application

2. Alcohols

- Ethanol; isopropanol; used at concentrations between 70 – 95%
- Denature proteins; disrupt membranes
- Kills vegetative cells of bacteria & fungi but not spores
- Used in disinfecting surfaces

3. Halogens

- Act as oxidizing agents; oxidize proteins & other cellular components
- **Chlorine compounds**
  - Used in disinfecting municiple water supplies
  - Sodium Hypochlorite (Chlorine Bleach) used at 10 - 20% dilution as benchtop disinfectant
  - Halazone tablets used by campers to disinfect water for drinking
- **Iodine Compounds**
  - Used as antiseptics for cleansing skin surfaces and wounds

4. Heavy Metals

- Mercury, silver, zinc, arsenic, copper ions
- Form precipitates with cell proteins
- At one time were frequently used medically as antiseptics but much of their use has been replaced by less toxic alternatives
- Examples: 1% silver nitrate was used as opthalmic drops in newborn infants to prevent gonorrhea; has been replaced by erythromycin or other antibiotics; copper sulfate used as algicide in swimming pools

5. Quaternary Ammonium Compounds

- Quaternary ammonium compounds are cationic detergents
- Denature proteins and disrupt membranes
- Used as disinfectants and skin antiseptics
- Examples: cetylpyridinium chloride, benzalkonium chloride

6. Aldehydes

- Formaldehyde and gluteraldehyde
- React chemically with nucleic acid and protein, inactivating them
- Aqueous solutions can be used as disinfectants

Thank you…