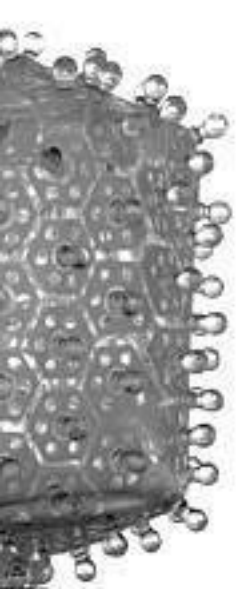


Microbiology

Lecture No: 3

Dr Name: Asem Shehabi

Sheet ☐ Slide ■

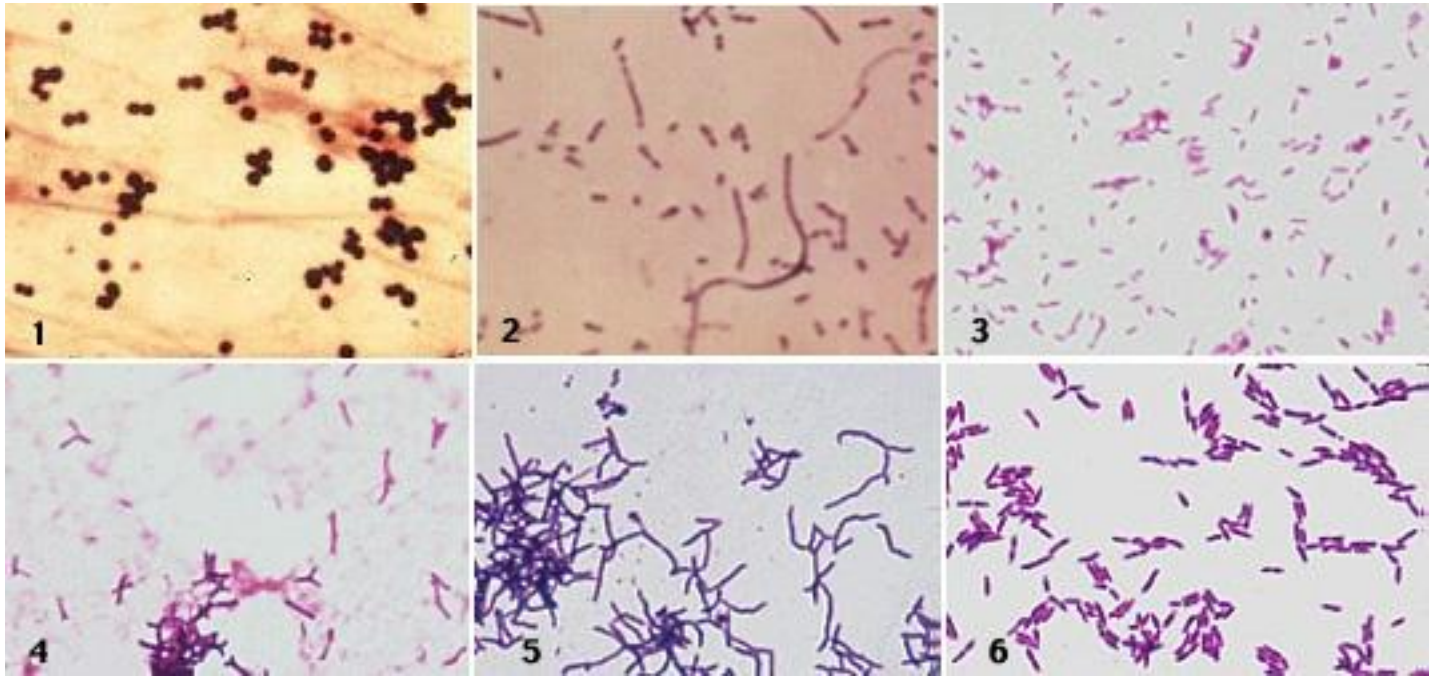
Mrym Ghuloom

Disinfection & Sterilization

By

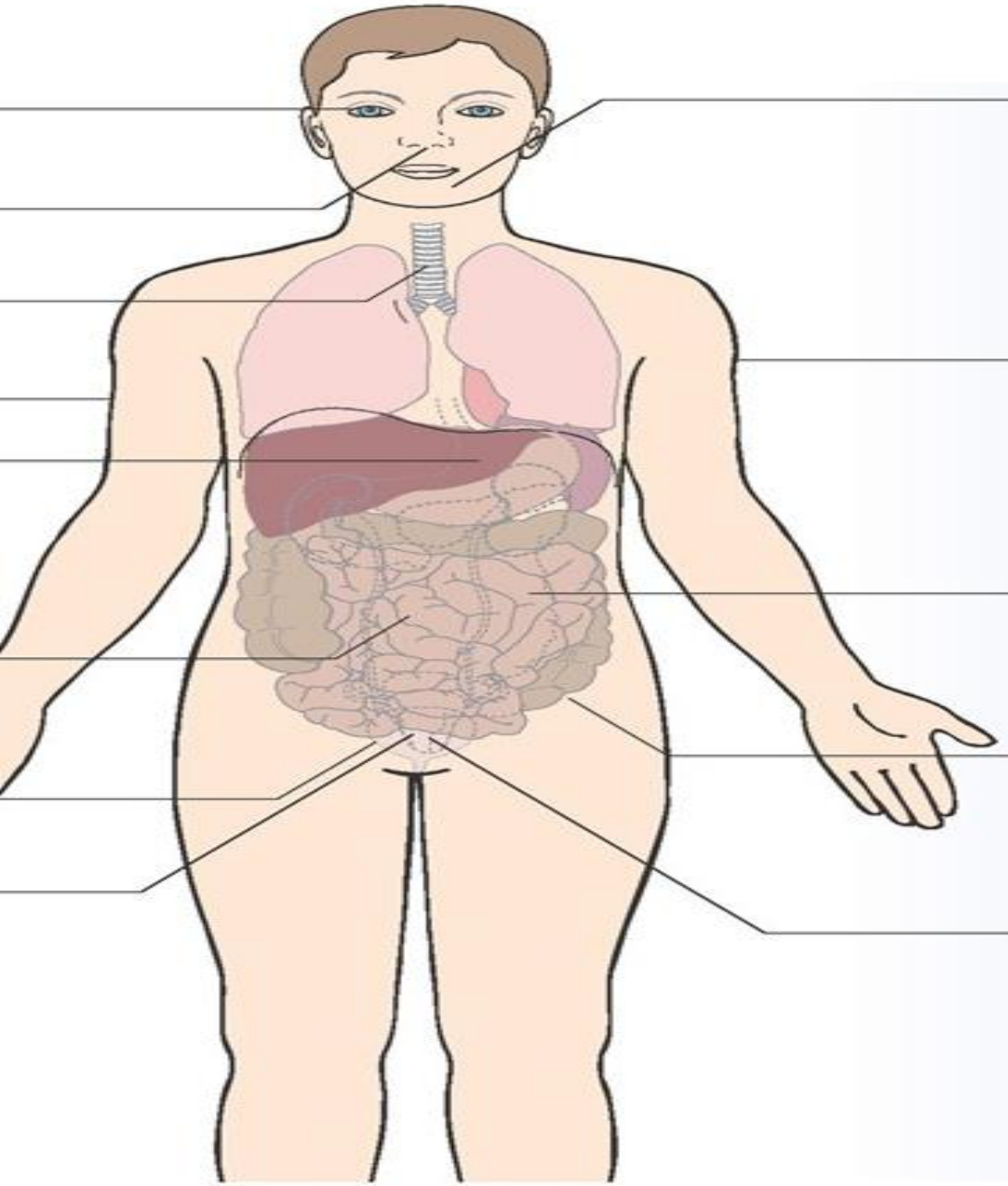
Prof. Dr. Asem Shehabi and Dr. Suzan Matar

Bacteria - Normal flora



Normal body Flora Human-1

- A Large variety of microorganisms colonize human body throughout its entire live.
- Human body has actually more bacterial cells than human cells. Harbors about 10^{14} bacteria, few yeast, rarely ectoparasites (Lice, dust mites) & viruses.
- A large amount of bacteria species (commensals) colonize intestines, body cavities, skin pores, sweat glands & air follicles..mostly Anaerobes (about 95%), **Facultative Anaerobes (5%).**



NORMAL FLORA

NASOPHARYNX
- Streptococci
- *Haemophilus*
- *Neisseria*
- Mixed anaerobes
- *Candida*
- *Actinomyces*

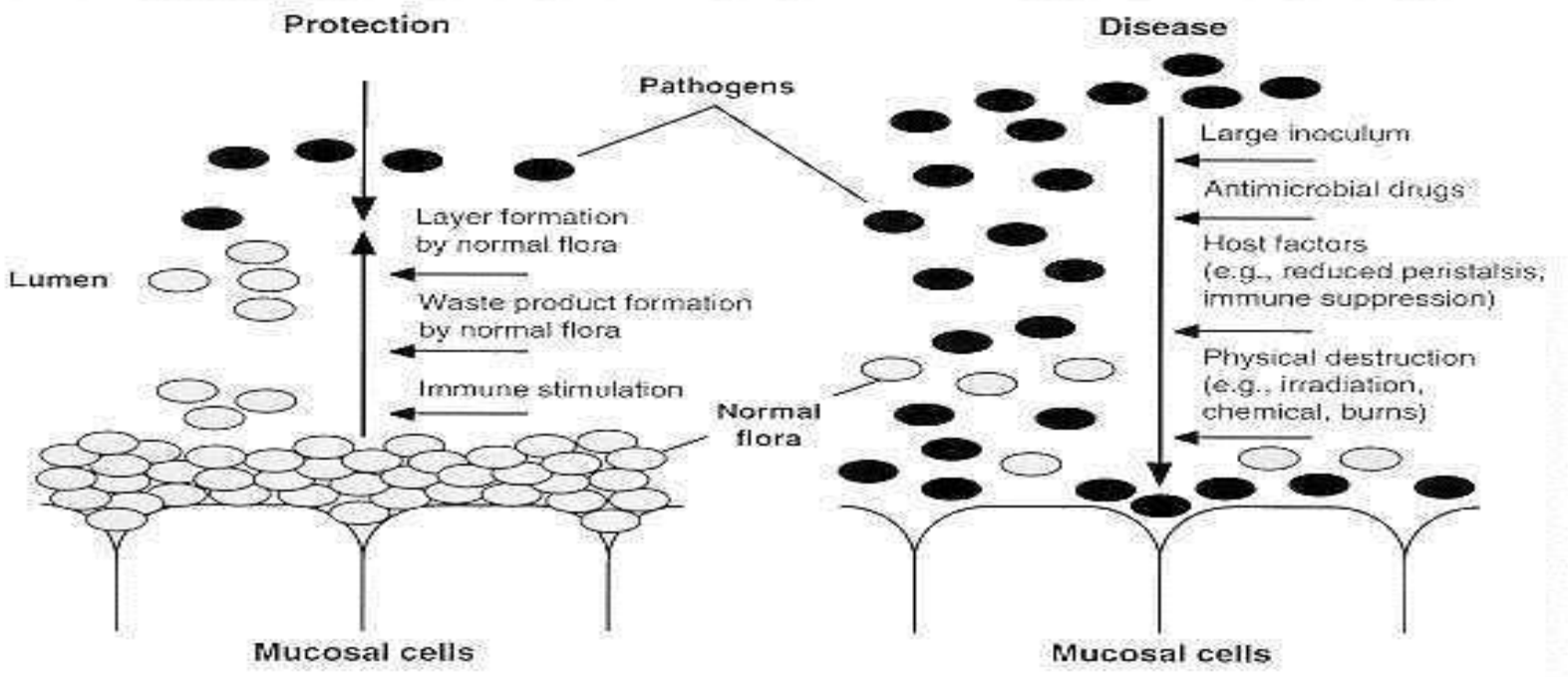
SKIN
- Staphylococci
- Streptococci
- Corynebacteria
- Proprionibacteria
- Yeasts

UPPER BOWEL
- Enterobacteriaceae
- Enterococci
- *Candida*

LOWER BOWEL
- *Bacteroides*
- Bifidobacteria
- *Clostridium*
- Peptostreptococci

VAGINA
- Lactobacilli
- Streptococci
- Corynebacteria
- *Candida*
- *Actinomyces*
- *Mycoplasma hominis*

- All normal flora competing with pathogens & prevent their adherence.
- Produce provitamins, inorganic acids, eliminating toxins & radicals, enhancing mucosal & body immunity



Mechanisms by which the normal flora competes with invading pathogens

Normal Flora -2

- **Skin Flora:** *Staphylococcus spp.* & *Propionobacterium* may cause localized inflammation.. Wounds.. Sepsis, Surgery
- **Oral Cavity and Nasopharyngeal Flora:** *Streptococcus spp., Neisseria spp., Corynebacterium spp. Haemophilus spp.* Protective against invasion of pathogenic organisms to some extent.
- **Intestinal Flora**: The colon may contain **10^9 to 10^{11}** bacteria per gram of feces. Mostly (about 95 %) are obligate anaerobes, *Bacteroides*, *Bifidobacterium*, Lactobacilli, Streptococci ,*Clostridia, Enterobacteriaceae* (*E. coli, Enterobacter, Klebsiella species*) & few Yeast

Normal Flora-3

- Urogenital Flora: The urogenital tract is normally sterile .. the vagina and the distal 1 cm of the urethra contain: *Lactobacillus* predominate in the vagina in jung women.. control acidity.. pH 4.5.. Prevent growth of few Yeast (Candida species).

- The **urethra** may contain predominantly skin microorganisms including: *Staphylococci*, *Streptococci*, *Diphtheroids*.

Physical Control of Microbial Growth

- Terminology:
- **Sepsis :** microbial presence in sterile body fluid/tissue/contamination, **Aspesis:** absence of contamination
- Antiseptic: process used to destroy microorganisms on living tissues, skin, mucosa, wound.
- **Disinfection/Disinfectant (sanitization):** Used for non-living objects to destroy microorganisms with up 99% killing effect.
- **Sterilization/Sterile:**100% Killing effect against all microorganisms.. Microbial contamination
- Mostly effect cell membrane permeability, damage proteins & nucleic acids of organisms.
- Killing vegetative & spore forming *bacteria, Fungi, Viruses, Parasites*

- Bacteriostatic.. Bactericidal .. Microbiocidal ..
- Refrigeration .. Deep Freezing .. Desiccation of Food

Moist heat: Boiling temperature 100 ^{0}C

causes irreversible coagulation of proteins found in microorganisms.

1 – 2 minutes of boiling destroyed most microorganisms, except spore-forming bacteria and few other viruses and parasites.

Methods to Control Microbial Growth

- Physical Methods
  - Heat Sterilization
    - Dry Heat
      - Incineration
    - Moist Heat
      - Boiling
      - Autoclaving
      - Pasteurization
  - Filtration
    - Air
    - Liquid
  - Radiation
    - UV Light
    - Ionizing Radiation

Physical Control of Microbial Growth-2

- **Sterilization Methods:**
  - **Direct Flaming ..Incineration.. Burning**
  - Dry Heat/ Hot-air Oven: 170-180 C / 2 Hours

    (200ºC, 1.5 hrs dry = 121ºC, 15 min moist)

    - e.g flasks, tubes, pipettes in microbiological laboratories.
  - Moist Heat/ Steam Under Pressure .. Autoclave .. 121C /15 PSI/ 15 Minutes
  - **Ionizing irradiation:** Cold sterilization

    High-Energy Electromagnetic Beams, Gamma Rays, Radioactive Cobalt 60,

    Disposable Plastic Wares, Pharmaceutical products, **Food..**

    All irradiation methods damage cellular DNA.

Ultraviolet-Autoclave

* **Filtration:** Liquids, using Nitrocellulose Membrane/ Pore Sizes 0.01-O.2um
* Pasteurization

- reduces number of heat sensitive pathogenic organisms

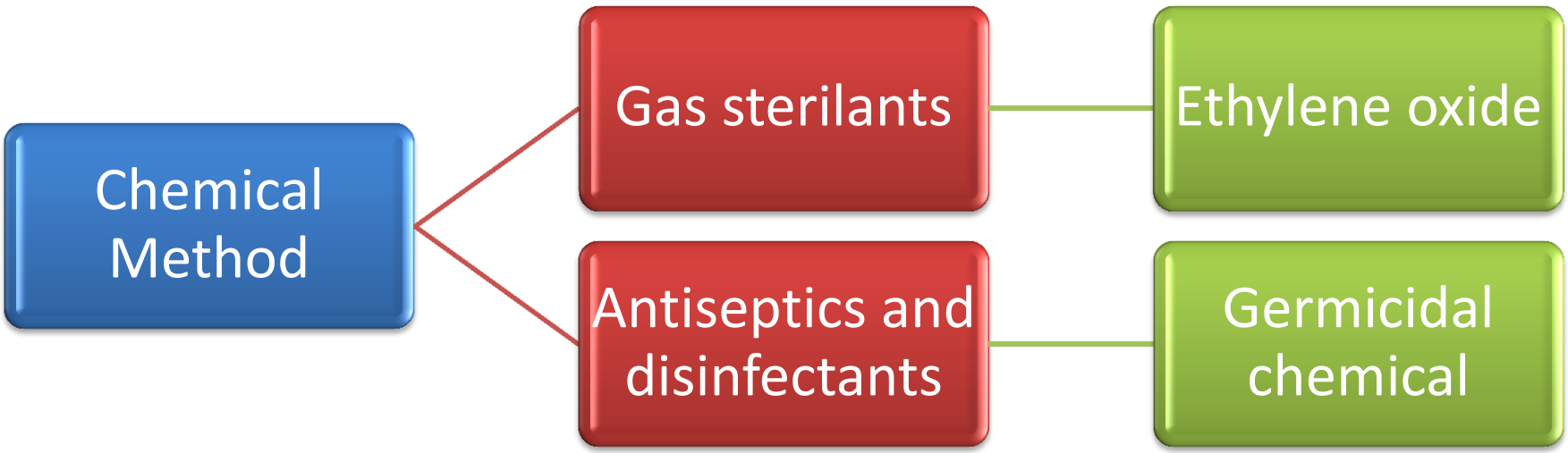
- widely used in milk and juices

- increases shelf life and does not alter Original pasteurization was **62ºC, 30** quality. **mins..** now: UHT-shorter time **72ºC, 15 secs**

UV Light: Non ionizing radiation. 240-280 nm, 12-24 Hours Exposure .. Damages the structure and function of nucleic acids

- Penetrate poorly- cannot penetrate even into liquid.
- Used to disinfect surfaces
- Can cause damage to human cells
- Germicidal lamps -kill or reduce the number of viable microorganisms to sterilize microbiological laboratories hospital operating rooms, and specific filling rooms in various industries

Chemical Microbial Control



Sterilization Gases

Alkylating Gases:

- Ethylene Oxide: is highly reactive and interact with many cell structures, highly toxic for human respiratory tract & flammable. Should be mixed with10% Co_2, N_2 before used. **4-12 Hours**, Fiber endoscopes, Heart-lung machine, Textiles, Disposable plastic article,
- Formaldehyde Gas ..Aqueous Solution 37% Formalin biopsies.. 2% Aqueous Glutaraldehyde is used to preserve tissue
- Patients room as gas vapor. Long Exposure Time (10-24 Hours). Highly toxic for human.

Chemical Control of Microbial Growth-1

- Disinfection Methods: For surgical scrub, cuts/ wound/ skin injury ointment, skin cleansing
- **Influencing Factors:**

- Presence of Organic Materials/ Contaminations..
- First Cleaning to decrease the concentration of Agent
- pH Medium, Contact Time

- **Disinfectant /Antiseptics Agents**
  - **Alcohols:** Ethanol/ Isopropanol (70-90% solution)
    coagulated enzymes and proteins and damage lipid membranes
  - **Aqueous Iodine** (3-5%) , Tincture Iodine (Alcohol-Iodine)
    Betadine / Povidone-iodine, 2 minutes
  - **Chlorhexidine.. Cetrimide.. Savlon**
  - All should be used for only external use.

- Water-Disinfection: **Chlorine Gas**, Na-Hypochlorite..dissolve Hypochlorous Acid (HOCl in water .. Release Active Cl^{ions}..

  with 2-3 PPM.. Kill most pathogens.

- Fecal E. coli.. Used as indicator of water contamination.. Safe drinking water must free of *E.coli*

- Oxidizing Agents:

- Ozone (O_3)..Disinfect Water .

- Hydrogen Peroxide (H_2O_2) Skin & wounds cleansing

- Other chemical agents used for disinfection of innate objects:
  - Phenol compounds.. Hexachlorophene / Dettol, Lysol
  - Organic Acids.. Sorbic & Benzoic Acid.. Food Preservation, Cosmetic.. For Control Molds/ Fungi, Bacteria
  - Detergents.. Surface-Active Agents.. Positive/negative charged ions.. Like Soaps, Wash-Powder, Hair-Shampoo

- **Hand washing: A simple way to prevent spread of infection and disease.**
- **Hand washing is a simple habit that can help keep you healthy.**
- **Good hand hygiene .. First step to protect yourself & others and control nosocomial infection.**