MICROBIOLOGY

CIMS 0121

By Jimmy Ongori

LECTURE ONE

INTRODUCTION AND HISTORY OF MICROBIOLOGY

Lectures on;

- Monday 10.00 – 12.00
- Tuesday 4-6pm

INTRODUCTION

- Microbiology is the study of microorganisms - organisms which are of microscopic dimensions.
- These organisms are too small to be perceived by the unaided human eye.
- If an object has a diameter of less than 0.1mm, the eye can not perceive it at all, and very little detail can be perceived in an object with a diameter of I mm.

INTRODUCTION Cont'

- Generally organisms with a diameter of 1 mm or less are microorganisms and fall into the broad domain of microbiology.
- Since most microorganisms are only a few thousandths of a millimetres in size they can only be seen with the aid of microscope
- These include protozoa, algae, fungi and bacteria. Viruses are ultramicroscopic and have an obligate parasitic relationship, but they still come under the domain of microbiology.

What is Microbiology?

- Micro - too small to be seen with the naked eye
- Bio - life
- Ology - study of

Organisms included in the study of Microbiology

1. Bacteria	• Bacteriology
2. Protozoans	• Protozoology
3. Algae	• Phycology
4. Parasites	• Parasitology
5. Yeasts and Molds	
• Fungi	• Mycology
6. Viruses	• Virology

EARLY HISTORY OF MICROBIOLOGY

- Historians are unsure who made the first observations of microorganisms, but the microscope was available during the mid-1600s, and an English scientist named **Robert Hooke** made key observations.
- In 1665 he observed strands of fungi among the specimens of cells he viewed.
- He proposed the Cell Theory - all living things are made up of cells

History of Microbiology

- In the 1674, **Anton van Leeuwenhoek** was 1st person to view microorganisms
- Made careful observations of microscopic organisms, which he called **animalcules.**
- Until his death in 1723, van Leeuwenhoek revealed the microscopic world to scientists of the day and is regarded as one of the first to provide accurate descriptions of protozoa, fungi, and bacteria.

Spontaneous generation

- Theory that life just “spontaneously” developed from non-living matter
- After van Leeuwenhoek died, the study of microbiology did not develop rapidly because microscopes were rare and the interest in microorganisms was not high.
- In those years, scientists debated the theory of **spontaneous generation**, which stated that microorganisms arise from lifeless matter such as beef broth.

History

- In 1668 the theory of spontaneous generation was disputed by **Francesco Redi**, who showed that fly maggots do not arise from decaying meat (as others believed)
- To prove this he covered the meat with gauze to prevent entry of flies. Hence they could not deposit their eggs. No maggots appeared

- Schleiden and Schwann
  - Formulated Cell Theory: *cells are the fundamental units of life and carry out all the basic functions of living things*
- Pasteur, FR and Tyndall, UK (1861)
  - Finally disproved Spontaneuous Generation.
- Joseph Lister, UK (1867)
  - Used phenol (carbolic acid) to disinfect wounds
  - First aseptic technique in surgery

- Robert Koch, (1876)
  - Postulates – Germ theory (1876)
  - Identified microbes that caused anthrax (1876), tuberculosis (1882) and cholera (1883)
  - Developed microbiological media & streak plates for pure culture (1881)
  - Gave rise to Koch's postulates

Koch's Postulates

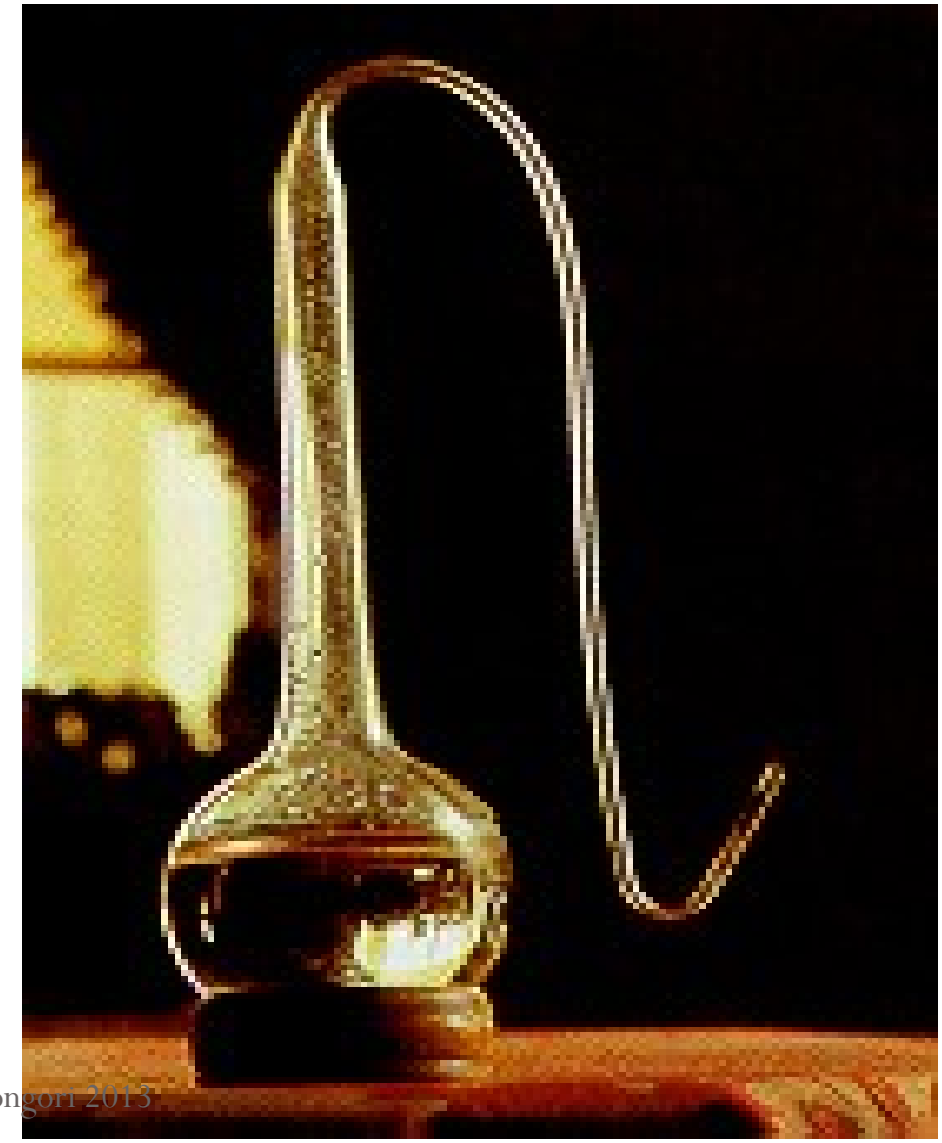
1. The specific causative agent must be found in every case of the disease.
2. The disease organism must be isolated from the lesions of the infected case and maintained in pure culture.
3. The pure culture, inoculated into a susceptible or experimental animal, should produce the symptoms of the disease.
4. The same bacterium should be re-isolated in pure culture from the intentionally infected animal.

Pasteur's Contribution to Microbiology

- Pasteur showed that microorganisms are not evenly distributed in the atmosphere and that their number varies from place to place.

- For this, he took a large number of sealed flasks containing boiled and cooled infusions and opened a few at a time for a short period at various places and resealed.

Pasteur designed special “swan-necked flasks” with a boiled meat infusion



Shape of flask allowed air in (vital force) but trapped dust particles which may contain microbes

- Out of the 20 flasks which he opened and resealed on a dusty road, 8 showed spoilage
- Out of the 20 that he opened on the top of a mountain, only five showed spoilage
- Out of 20 that he opened near a glacier, only one showed spoilage.
- During the short time that the flasks were open, air had rushed into the flasks carrying along with it the microorganisms. After resealing and incubation, only those flasks which got the microbes from the air showed growth and spoilage.

- From these experiments he concluded that the air contained microbes and the number of microorganisms in the atmosphere varied from place to place
- He also made an intensive study of the beer and wine manufacturing processes and causes of souring and spoilage of beer and wines. He found that wine spoilage was caused by the growth of undesirable contaminating microbes which produced the so called "disease".

- The solution to this problem lay in preventing the growth of undesirable organisms.
- After considerable experimentation Pasteur showed that wine did not undergo spoilage if it was held for a few minutes at 50 to 60°C. In the same way, he found that beer could also escape the “disease” by heating to 50-55° C.
- This gave rise to the new process of preserving wine, fruit juices, milk etc., and was called "Pasteurization".

The Germ theory of Disease

1835: Agostino Bassi showed a silkworm disease was caused by a fungus.

1865: Pasteur believed that another silkworm disease was caused by a protozoan.

1840s: Ignaz Semmelwise advocated hand washing to prevent transmission of puerperal fever

1860s: Joseph Lister used a chemical disinfectant to prevent surgical wound infections

1796: Edward Jenner inoculated a person with cowpox virus. The person was then protected from smallpox.

- Called vaccination from *vacca* for cow
- The protection is called immunity

Chemotherapy – treatment with chemicals

- 1910: Paul Ehrlich developed a synthetic arsenic drug, salvarsan, to treat syphilis.
- 1930s: Sulfonamides were synthesized
- 1928: Alexander Fleming discovered the first antibiotic - penicillin, that killed *Staphylococcus aureus*.

DEFINITION OF SOME TERMS

i. Pathogen – disease causing microorganism
ii. Infection – the process of transmission of disease by a disease causing MO
iii. Pathogenesis – process by which disease starts and develops within the body

Benefits of MOs

- Maintain balance of environment (microbial ecology)
- Basis of food chain
- Nitrogen fixation
- Photosynthesis
- Digestion, synthesis of vitamins
- Manufacture of food and drink, drugs
- Genetic engineering
- Synthesis of chemical products
- Recycling sewage
- Bioremediation: use microbes to remove toxins (oil spills)
- Use of microbes to control crop pests
- Normal microbiota

LECTURE TWO

BACTERIAL GROWTH

GROWTH

- Growth is the orderly increase in the sum of all the components of an organism.
- Increase in size that results when a cell takes up water or deposits lipid or polysaccharide is not true growth.
- Cell multiplication is a consequence of growth; in unicellular organisms, growth leads to an increase in the number of individuals making up a population or culture.

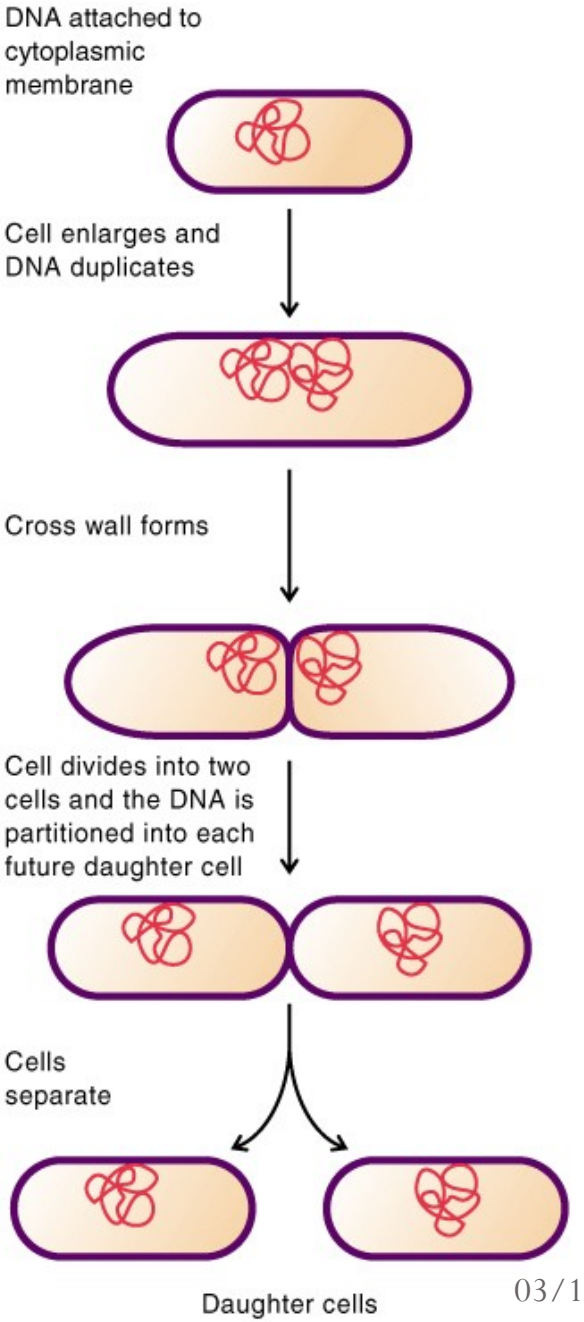
GROWTH Cont'

- Microbial concentrations can be measured in terms of;
  - cell concentration (the number of viable cells per unit volume of culture) or
  - biomass concentration (dry weight of cells per unit volume of culture).
- Culture - is a method of propagating/multiplying microbial (living tissue cells) in media conducive to their growth

GROWTH Cont'

- Most Bacteria Reproduce by **Binary Fission**
  - The cell doubles in size
  - Replicates the chromosome (DNA)
  - Forms a septum in the center
  - Synthesizes a Cell Wall at the Septum
  - Daughter cells separate.

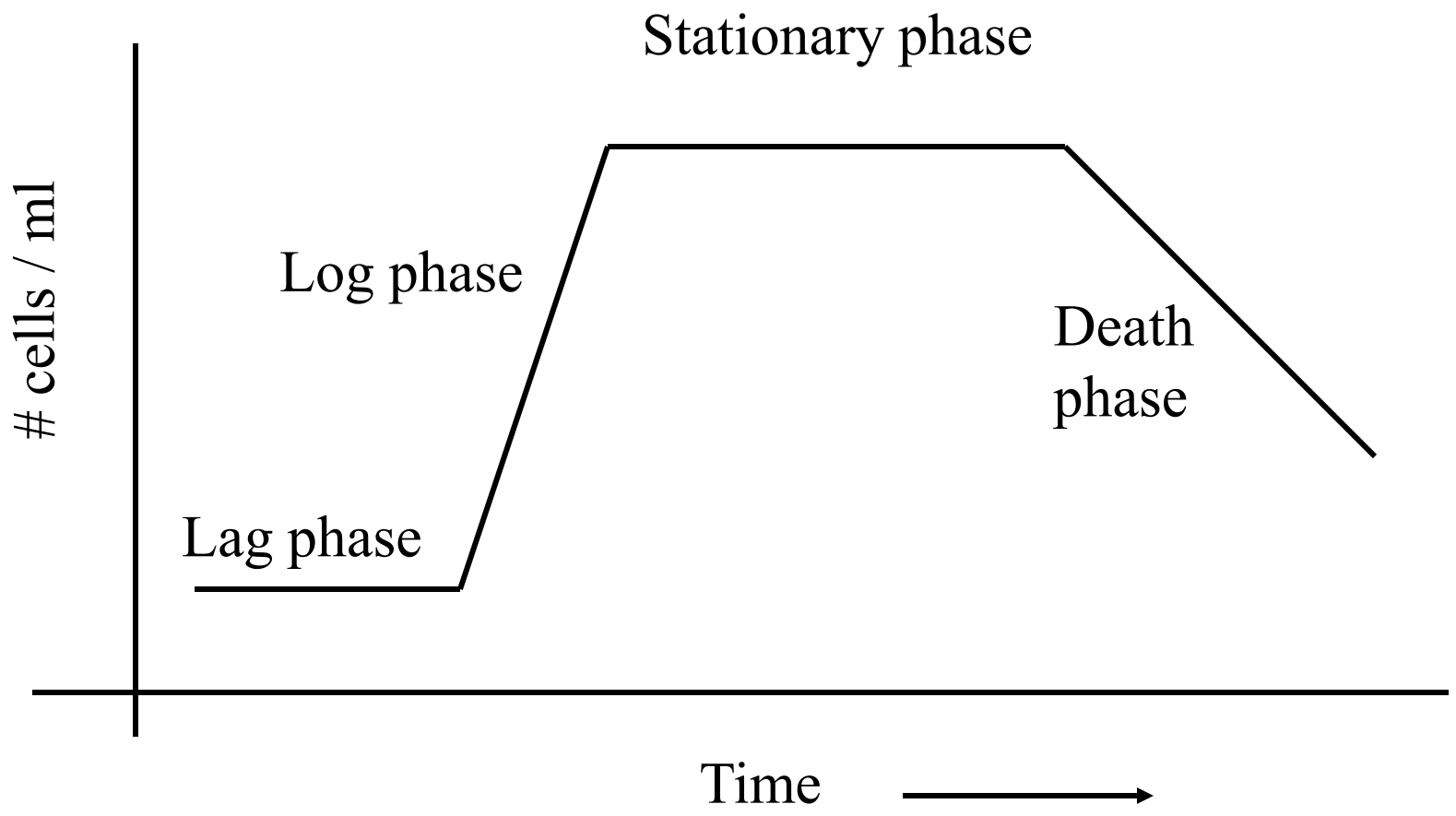
Binary fission



GROWTH Cont'

- All microorganisms undergo similar growth patterns.
- Each growth Curve has 4 Phases
  - Lag phase
  - Logarithmic phase
  - Stationary phase
  - Death phase

Microbial Growth Curve



1. LAG PHASE

- The lag phase represents a period during which the cells, depleted of metabolites and enzymes as the result of the unfavorable conditions that existed at the end of their previous culture history, adapt to their new environment.
- Enzymes and intermediates are formed and accumulate until they are present in concentrations that permit growth to resume.

LAG PHASE

- If the cells are taken from an entirely different medium, it often happens that they are genetically incapable of growth in the new medium.
- In such cases a long lag may occur, representing the period necessary for a few mutants in the inoculum to multiply sufficiently for a net increase in cell number to be apparent.

2. LOGARITHMIC/EXPONENTIAL PHASE

- During the exponential phase, the cells are in a steady state.
- New cell material is being synthesized at a constant rate, but the new material is itself catalytic, and the mass increases in an exponential manner. This continues until one of two things happens: either one or more nutrients in the medium become exhausted, or toxic metabolic products accumulate and inhibit growth.

2. LOG PHASE

- For aerobic organisms, the nutrient that becomes limiting is usually oxygen.
- Population doubles every generation
- Microbes are sensitive to adverse conditions e.g.
  - Antibiotics
  - Anti-microbial agents

3. STATIONARY PHASE

- Eventually, the exhaustion of nutrients or the accumulation of toxic products causes growth to cease completely.
- In most cases, however, cell turnover takes place in the stationary phase: There is a slow loss of cells through death, which is just balanced by the formation of new cells through growth and division. When this occurs, the total cell count slowly increases although the viable count stays constant.

3. STATIONARY PHASE

- Cells begin to encounter environmental stress due;
  - Lack of nutrients
  - Lack of water
  - Not enough space
  - Metabolic wastes
  - Oxygen
  - pH

4. DEATH PHASE (PHASE OF DECLINE)

- After a period of time in the stationary phase, which varies with the organism and with the culture conditions, the death rate increases until it reaches a steady level.
- Death due to limiting factors in the environment
- In most cases the rate of cell death is much slower than that of exponential growth.
- After the majority of cells have died, the death rate decreases drastically, so that a small number of survivors may persist for months or even years. This persistence may in some cases reflect cell turnover, a few cells growing at the expense of nutrients released from cells that.

REQUIREMENTS FOR GROWTH

- Growth refers to increase in number of cells
- In terms of a single cell it is seen as an increase in size and mass over time
- Growth requirements can be divided into;
  1. Physical – temperature, pH, osmotic pressure
  2. Chemical – water, micro and macro-nutrients, organic growth factors

TEMPERATURE

- It is one of the most important environmental variables affecting microbial growth
- Every MO possesses a characteristic range of temperatures over which it can grow
- For a particular MO there is a minumum, maximum and optimum temperature
  - Minimum – lowest temp' at which the spp. Can grow
  - Optimum – temp' at which spp grows best
  - Maximum – highest temp' at which growth is possible

TEMPERATURE

- **Psychrophilic** 0-20^0 C but grow best at low temperatures (15–20 °C)
- **Mesophilic** forms grow best at 30–37 °C
- **Thermophilic** forms grow best at 50–60 °C.
- Some organisms are **hyperthermophilic** and can grow above the temp' of boiling water, which exists under high pressure in the depths of the ocean.
- Most organisms are mesophilic; 30 °C is optimal for many free-living forms, and the body temperature of the host is optimal for symbionts of warm-blooded animals.

Psychrophiles

- Some can grow at 0^0C, but will not grow beyond 20^0 C
- Some, optimum is 20-30^0 C – common in food spoilage because can grow at refrigerator temp'

pH

- Every organism has a range of pH over which growth is possible and an optimal pH
- Most bacteria (**neutralophiles**) grow between pH 6.5 - pH 7.5
- **Acidophilic** bacteria – grow in acidic pH eg Lactobacillus which produce acid. Most fungi grow at 1.5-2.0
- **Alkalophiles** – can grow upto pH10.5

OSMOTIC PRESSURE

- **Hypertonic** environments, increase salt or sugar, cause plasmolysis
- **Extreme or obligate halophiles** require high osmotic pressure
- **Facultative halophiles** tolerate high osmotic pressure

The Requirements for Growth: Physical Requirements

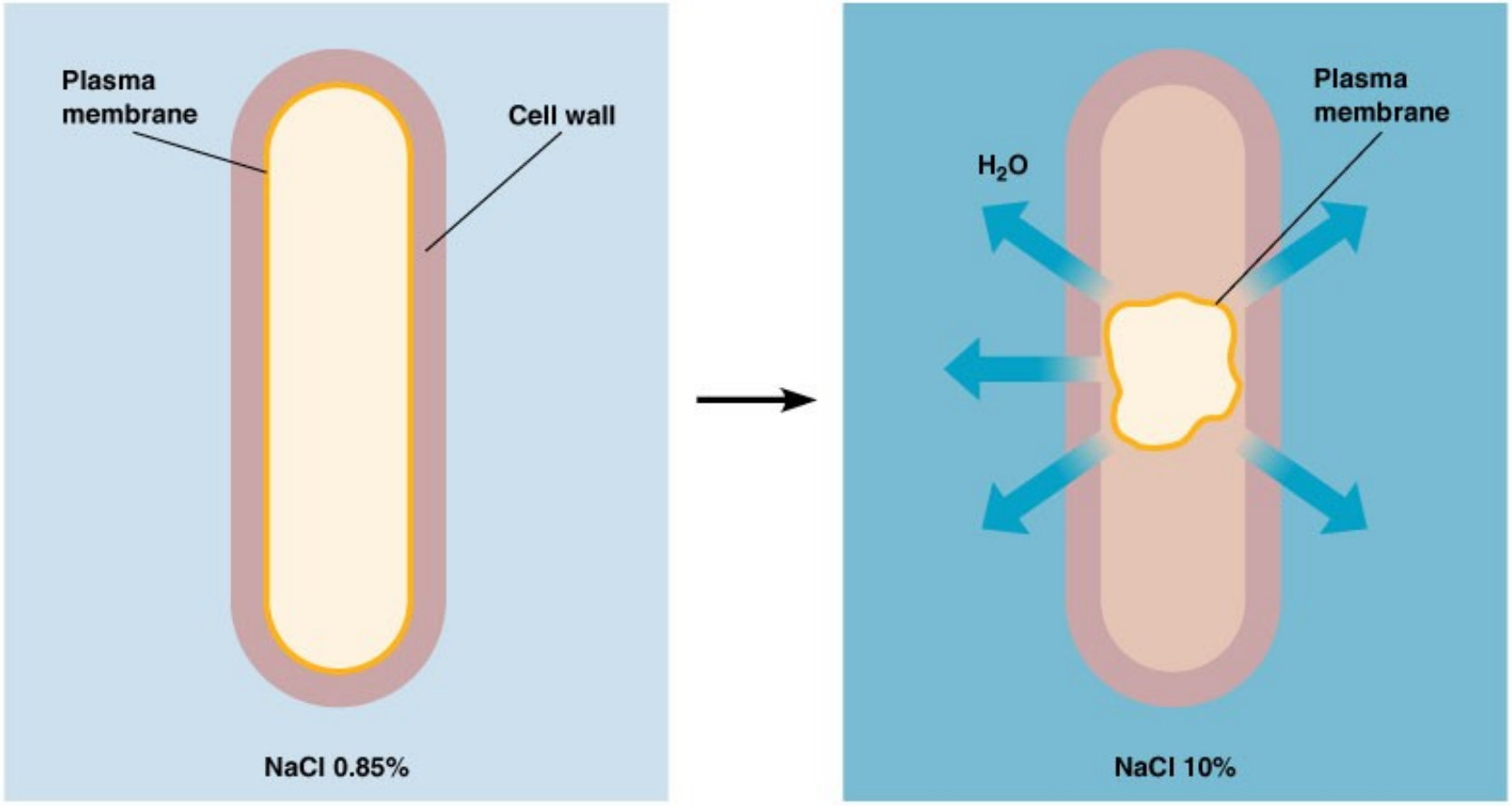


Figure 6.4

OXYGEN

- Many forms of life require oxygen for aerobic respiration
- Some forms of oxygen can actually be toxic
- Metabolism, UV light, chemical reactions can create toxic forms of oxygen
- Aerobes have enzymes to detoxify toxic forms of oxygen

Toxic Forms of Oxygen

- Singlet oxygen: O boosted to a higher-energy state
- Superoxide free radicals: O_2^-

$$O_2^- + 2\,H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2$$

- Peroxide anion: O_2^{2-}

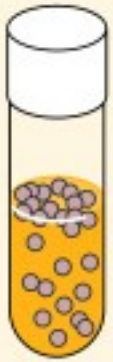
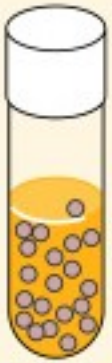
$$2\,H_2O_2 \xrightarrow{\text{catalase}} 2\,H_2O + O_2$$

$$H_2O_2 + 2\,O^+ \xrightarrow{\text{peroxidase}} 2\,H_2O$$

OXYGEN

- Obligate aerobes – require O_2 to survive
- Facultative anaerobes – can grow in the absence of O_2 eg *E. coli*
- Obligate anaerobes – unable to use molecular O_2 eg *Clostridia*
- Microaerophilic – aerobic but grow only in O_2 concentrations lower than those in the air

Oxygen (O_2)

obligate aerobes	Faultative anaerobes	Obligate anaerobes	Aerotolerant anaerobes	Microaerophiles
				

ASSIGNMENT

- Write short notes on the following;
  - Chemical requirements for microbial growth eg carbon, nitrogen, sulphur etc
  - Classify MOs according to requirements eg autotrophs etc

LECTURE 3

CULTURE MEDIA

- **Culture** is the term given to microorganisms that are cultivated in the lab for the purpose of studying them.

- **Medium** is the term given to the combination of ingredients that will support the growth and cultivation of microorganisms by providing all the essential nutrients required for the growth (that is, multiplication) in order to cultivate these microorganisms in large numbers to study them.

- Organisms have varying needs hence, culture media have also been formulated with different ingredients.
- Culture media may be found as;
  - liquid (called broth)
  - semi-solid
  - solid.
- Media are solidified by the addition of solidifying agents such as agar (inert compound).
- Varying the concentration of agar will yield varying degrees of solidification.

- Culture media may be classified as:
  1. **Synthetic** media (**Defined**)
  2. **Complex** (Non-synthetic) media

- Synthetic media contain only ingredients for which a complete **chemical formula is known**.

- Complex media contain at least one ingredient for which a **chemical formula is not known** (such as milk, egg, malt, animal tissues)

- Culture media can also be classified based on the function they perform in determining various characteristics of organism that are able to grow on/in them
- e.g. **Differential, Selective media**.

- The primary function of culture media is to be able to grow particular organisms on/in them.
- Media should be devoid of any other living organisms.
- This is done through the process of **sterilization** (a process by which all living organisms and their spore forms are killed and the medium is made sterile)
- Sterilized through the process of **autoclaving** (using high temperatures that will kill all living organisms under **increased pressure** for specified periods of time – in an appliance called the **autoclave**)

Types of media

- For the cultivation of bacteria, a commonly used medium is **nutrient broth**, a liquid containing proteins, salts, and growth enhancers that will support many bacteria.
- To solidify the medium, an agent such as agar is added. Agar is a polysaccharide that adds no nutrients to a medium, but merely solidifies it. The medium that results is **nutrient agar.**
- Very few bacteria can decompose agar

COMPLEX MEDIA

- Many media for microorganisms are complex, reflecting the growth requirements of the microorganisms.
- Usually contain complex materials of biological origin eg milk, blood or yeast extract
- For instance, most fungi require extra carbohydrate and an acidic environment for optimal growth. The medium employed for these organisms is **potato dextrose agar**, also known as.
- For protozoa, liquid media are generally required, and for rickettsiae and viruses, living tissue cells must be provided for best cultivation.

Selective Media

- Inhibits the growth of some bacteria while selecting for the growth of others eg

1. Brilliant Green Agar

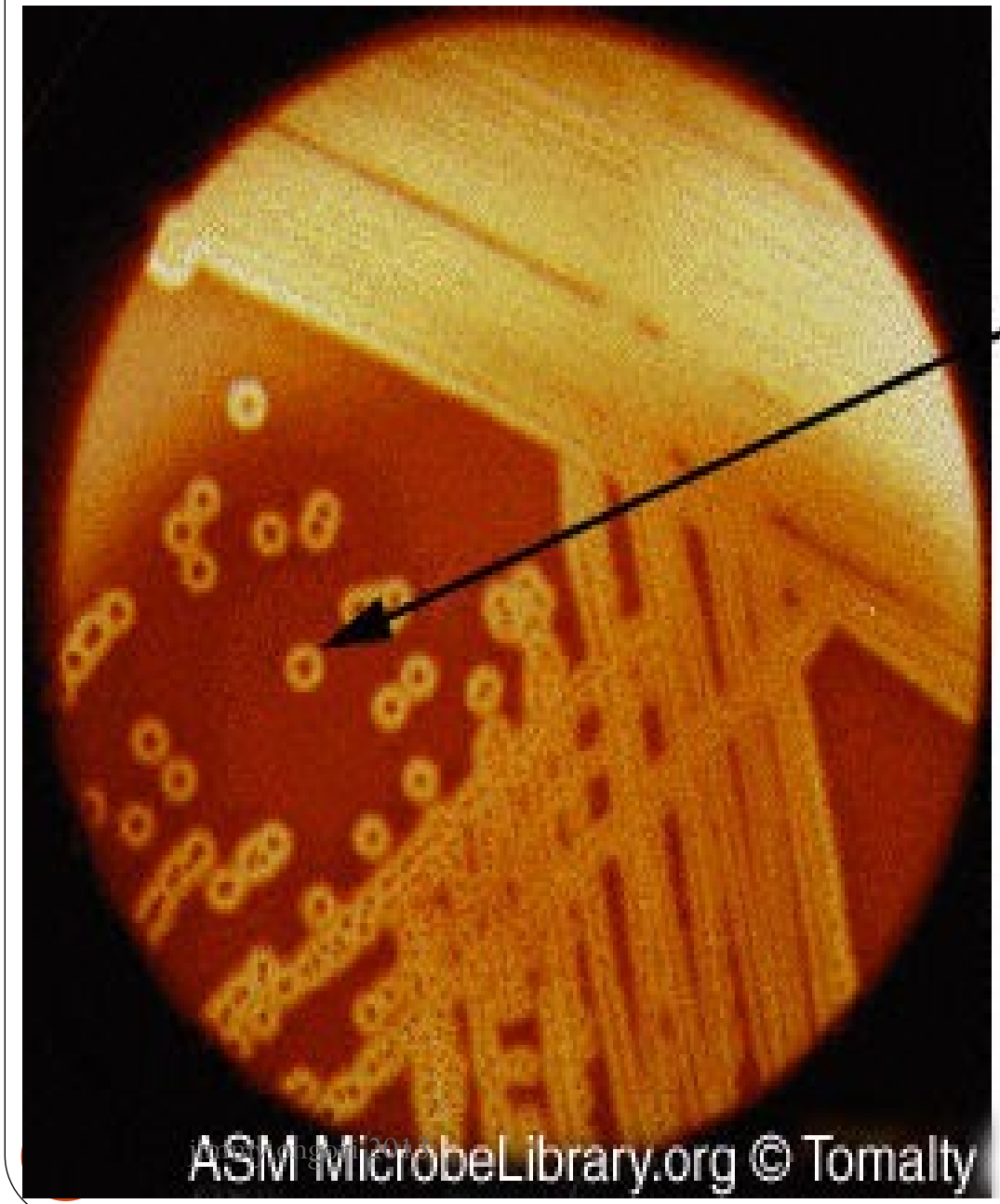
- dyes inhibit the growth of Gram (+) bacteria
- selects for Gram (-) bacteria
- Most G.I.T infections are caused by Gram (-) bact.

2. EMB (Eosin Methylene Blue)

- dyes inhibit Gram (+) bacteria
- selects for Gram (-) bacteria
- G.I. Tract infections caused by Gram (-) bacteria

Differential Media

- Differentiates between different organisms growing on the same plate
- Example:
  - Blood Agar medium (TSA with 5% sheep blood)
    - used to differentiate different types of *Streptococci*
      - Alpha-Hemolysis- incomplete RBC lysis
      - Beta Hemolysis- complete RBC lysis
      - Gamma Hemolysis- no RBC lysis



Note the clear zone of beta-hemolysis surrounding the *Streptococcus* colonies when grown on blood agar.

Selective and Differential Media

Mannitol Salt Agar

- used to identify *Staphylococcus aureus*
- High salt conc. (7.5%) inhibits most bacteria
- Sugar - Mannitol
- pH Indicator (Turns Yellow when acidic)

MacConkey's Agar

- used to identify *Salmonella*
- Bile salts and crystal violet (inhibits Gram (+) bact.)
- Sugar - lactose
- pH Indicator
- Many Gram (-) enteric non-pathogenic bacteria can ferment lactose, *Salmonella* can not

Enrichment Broths

- "Encourage" the growth of a particular type of microbe;
- Addition of "nutrients" enrich for microbial group of interest eg
  - Cellulose broth- enriches for microbes which degrade cellulose
  - Petroleum Broth- enriches for microbes which could eat an oil spill.

Bacterial Morphology Arrangement

1. Bacilli
   a. Streptobacilli
   b. Bacilli

2. Cocci
   a. Cocci
   b. Doplococci
   c. Streptococci
   d. Staphylococci

Bacterial Morphology Arrangement

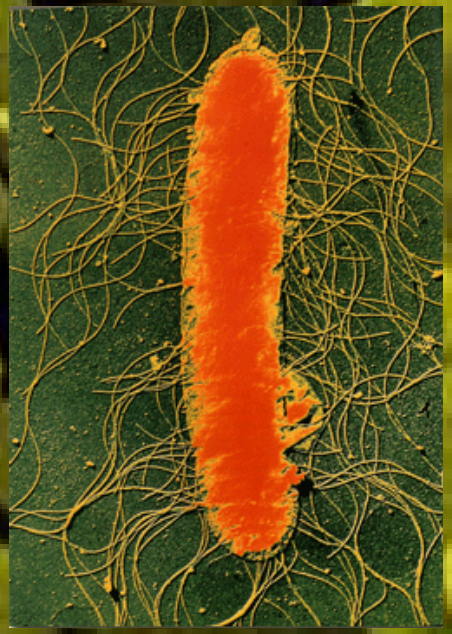
3 Spiral

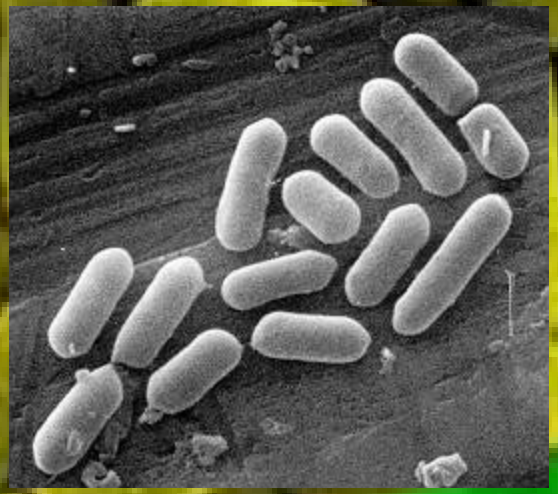
a. Vibrio

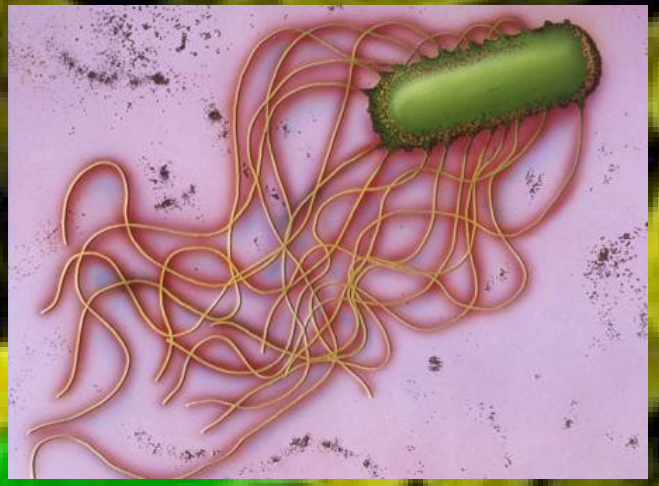
b. Spirillum

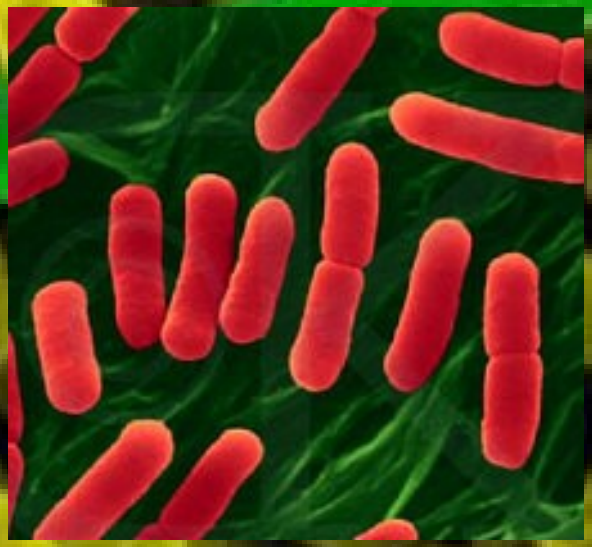
c. Spirochete

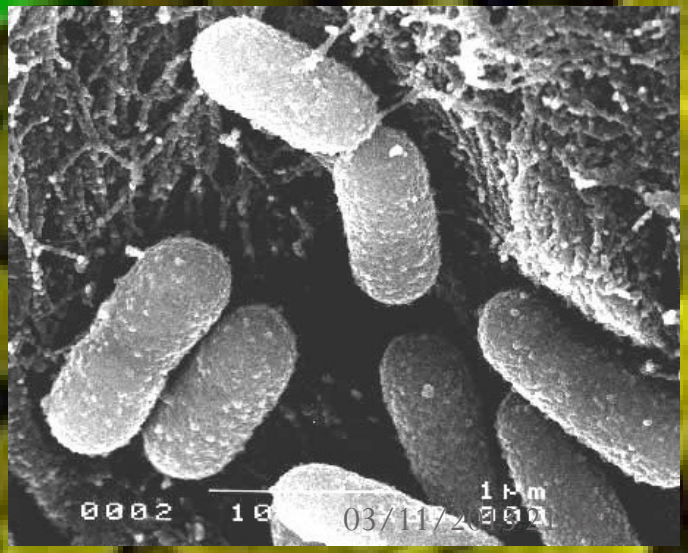
Rod-Shaped Bacteria









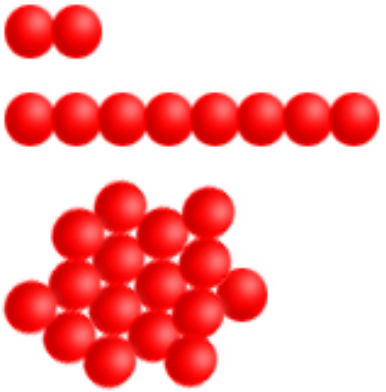


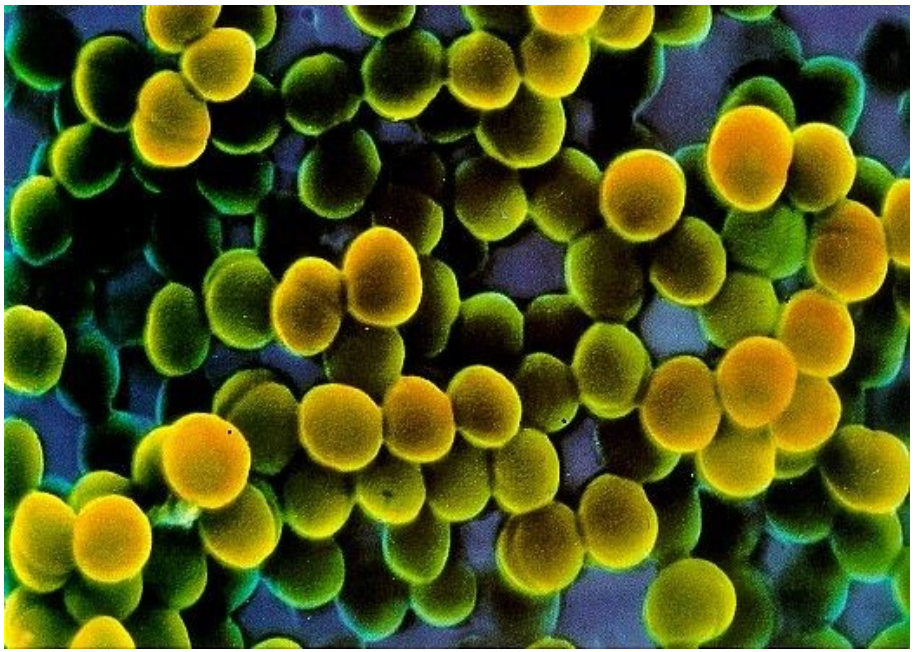
Spherical Bacteria

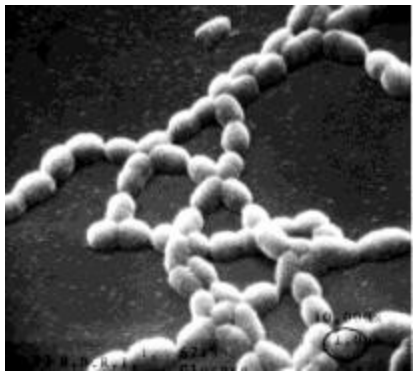
Diplococcus

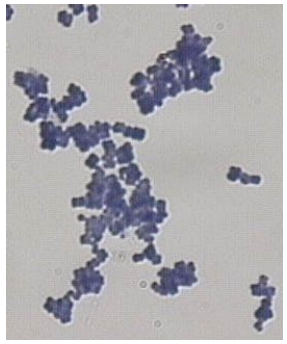
Streptococcus

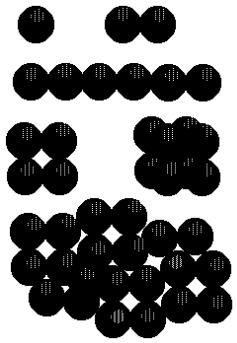
Staphylococcus



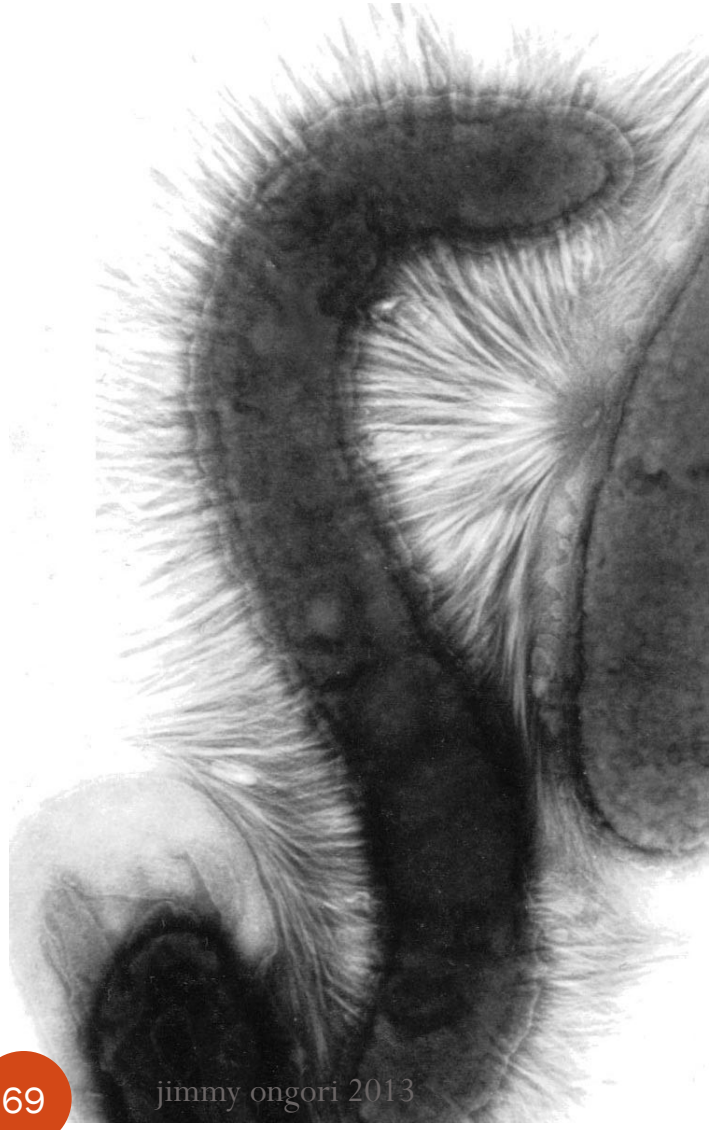




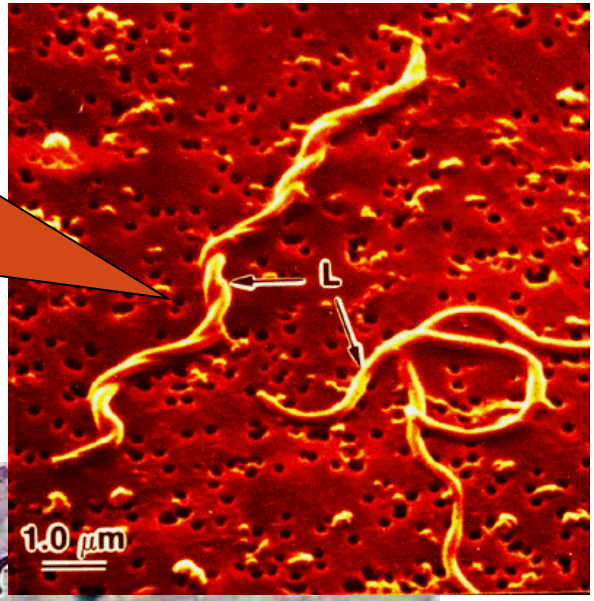


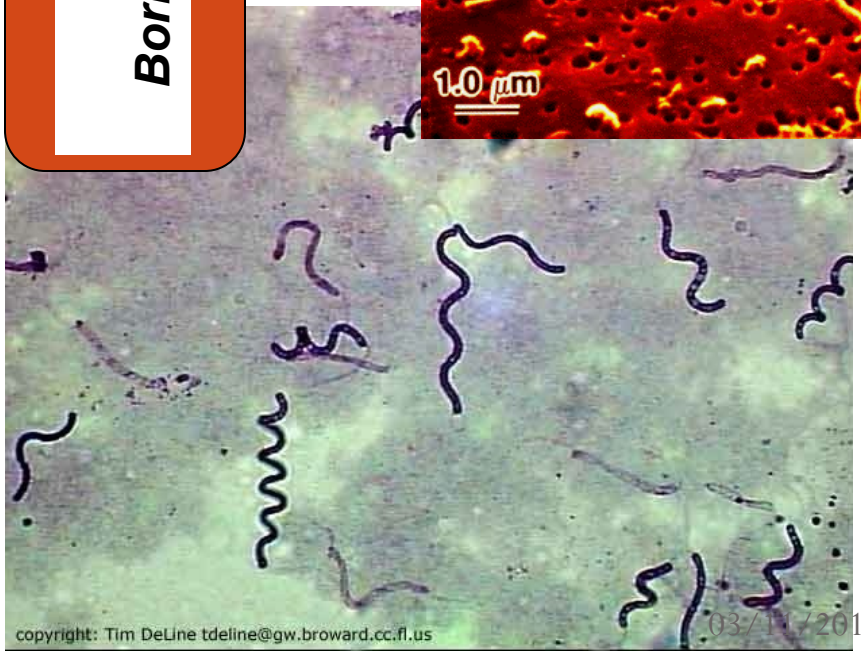


Spiral-Shaped Bacteria



Spirochete:
Borrelia burgdorferi





METHODS OF STUDY

OPTICAL METHODS

1. Light microscope
   a. Bright field microscope
   b. Phase contrast microscope
   c. Dark field microscope
2. Fluorescence Microscope
3. Differential Interference Contrast (DIC) Microscope
4. The Electron Microscope
5. Confocal Scanning Laser Microscope

1. The Light Microscope

- The resolving power of the light microscope under ideal conditions is about half the wavelength of the light being used. (**Resolving power** is the distance that must separate two point sources of light if they are to be seen as two distinct images.)
- The useful magnification of a microscope is the magnification that makes visible the smallest resolvable particles.
- Several types of light microscopes are commonly used in microbiology:

a. Bright-Field Microscope

- Is most commonly used in microbiology and consists of two series of lenses **(objective** and **ocular lens)**
- Generally uses a 100-power objective lens with a 10-power ocular lens, thus magnifying the specimen 1000 times. Particles 0.2 μ in diameter are magnified to about 0.2 mm and so become clearly visible.
- Specimens are rendered visible because of differences in **contrast** between them and the surrounding medium.
- Many bacteria are difficult to see well because of their lack of contrast with the surrounding medium.
- Dyes (stains) can be used to stain cells or their organelles and increase their contrast so that they can be more easily seen in the bright-field microscope.

b. Phase Contrast Microscope

- Was developed to improve contrast differences between cells and surrounding medium, making it possible to see living cells without staining them; with bright-field microscopes, killed and stained preparations must be used.
- Takes advantage of the fact that light waves passing through transparent objects, such as cells, emerge in different phases depending on the properties of the materials through which they pass.
- This effect is amplified by a special ring in the objective lens of a phase contrast microscope, leading to the formation of a dark image on a light background.

c. Dark-Field Microscope

- Light microscope in which the lighting system has been modified to reach the specimen from the sides only.
- This creates a "dark field" that contrasts against the highlighted edge of the specimens
- Resolution by dark-field microscopy is quite high. Thus, this technique has been particularly useful for observing organisms such as *Treponema pallidum*, a spirochete which is less than 0.2 µm in diameter and therefore cannot be observed with a bright-field or phase contrast microscope

FLUORESCENCE MICROSCOPE

- Used to visualize specimens that **fluoresce -** the ability to absorb short wavelengths of light (ultraviolet) and give off light at a longer wavelength (visible).
- Some organisms fluoresce naturally because of the presence within the cells of naturally fluorescent substances such as chlorophyll. Those that do not naturally fluoresce may be stained with a group of fluorescent dyes called **fluorochromes.**

- Widely used in clinical diagnostic microbiology eg the fluorochrome auramine O, which glows yellow when exposed to ultraviolet light, is strongly absorbed by *Mycobacterium tuberculosis*, When applied to a specimen containing *M tuberculosis* and exposed to ultraviolet light, the bacterium can be detected by the appearance of bright yellow organisms against a dark background.

- The principal use of fluorescence microscopy is a diagnostic technique called the **fluorescent-antibody (FA) technique** or **immunofluorescence.** In this technique, specific antibodies (eg, antibodies to *Legionella pneumophila*) are chemically labeled with a fluorochrome
- If the specimen contains *L pneumophila*, the fluorescent antibodies will bind to antigens on the surface of the bacterium, causing it to fluoresce when exposed to ultraviolet light.

Differential Interference Contrast (DIC) Microscope

- **DIC** microscopes employ a polarizer to produce polarized light. The polarized light beam passes through a prism that generates two distinct beams; these beams pass through the specimen and enter the objective lens where they are recombined into a single beam.
- Because of slight differences in refractive index of the substances each beam passed through, the combined beams are not totally in phase but instead create an interference effect, which intensifies subtle differences in cell structure. Structures such as spores, vacuoles, and granules appear three dimensional.
- DIC microscopy is particularly useful for observing unstained cells because of its ability to generate images that reveal internal cell structures that are less apparent by bright-field techniques.

The Electron Microscope

- The high resolving power of the electron microscope has enabled scientists to observe the detailed structures of prokaryotic and eukaryotic cells.
- Two types of electron microscopes in general use: the **transmission electron microscope (TEM),** and the **scanning electron microscope (SEM).**
- The TEM was the first to be developed and employs a beam of electrons projected from an electron gun and directed or focused by an electromagnetic condenser lens onto a thin specimen.
- TEM can resolve particles 0.001 m apart. Viruses, with diameters of 0.01–0.2 m, can be easily resolved.
- The SEM is useful for providing three-dimensional images of the surface of microscopic objects.

Confocal Scanning Laser Microscope

- A laser beam is bounced off a mirror that directs the beam through a scanning device. Then the laser beam is directed through a pinhole that precisely adjusts the plane of focus of the beam to a given vertical layer within the specimen.
- By precisely illuminating only a single plane of the specimen, in a relatively thick specimen, various layers can be observed by adjusting the plane of focus of the laser beam.
- Cells are often stained with fluorescent dyes to make them more visible. Alternatively, false color images can be generated by adjusting the microscope in such a way as to make different layers take on different colors. The CSLM is equipped with computer software to assemble digital images for subsequent image processing.

STERILIZATION AND DISINFECTION

Sterilization and Disinfection

Sterilization is defined as the process where all the living microorganisms, including bacterial spores are killed.

- Sterilization can be achieved by physical, chemical and physiochemical means.

Disinfection: Reducing the number of pathogenic microorganisms to the point where they no longer cause diseases. Usually involves the removal of vegetative pathogens

- Chemicals used in disinfection are called **disinfectants**.
- Not all disinfectants can kill all microorganisms. Some methods of disinfection such as filtration do not kill bacteria, they separate them out.

Sterilization and Disinfection

- Sterilization is an absolute condition while disinfection is not. The two are not synonymous.

Decontamination is the process of removal of contaminating pathogenic microorganisms from articles by a process of sterilization or disinfection.

- It is the use of physical or chemical means to remove, inactivate, or destroy living organisms on a surface so that the organisms are no longer infectious.

Sterilization and Disinfection

Asepsis: is the employment of techniques (eg gloves, air filters, uv rays etc) to achieve microbe-free environment.

Antisepsis: is the use of chemicals (antiseptics) to make skin or mucus membranes free of pathogenic microorganisms.

Bacteriostasis: is a condition where the multiplication of the bacteria is inhibited without killing them.

Bactericidal: Chemicals that can kill or inactivate bacteria. Have various names such as bactericidal, virucidal, fungicidal, microbicidal, sporicidal, tuberculocidal or germicidal.

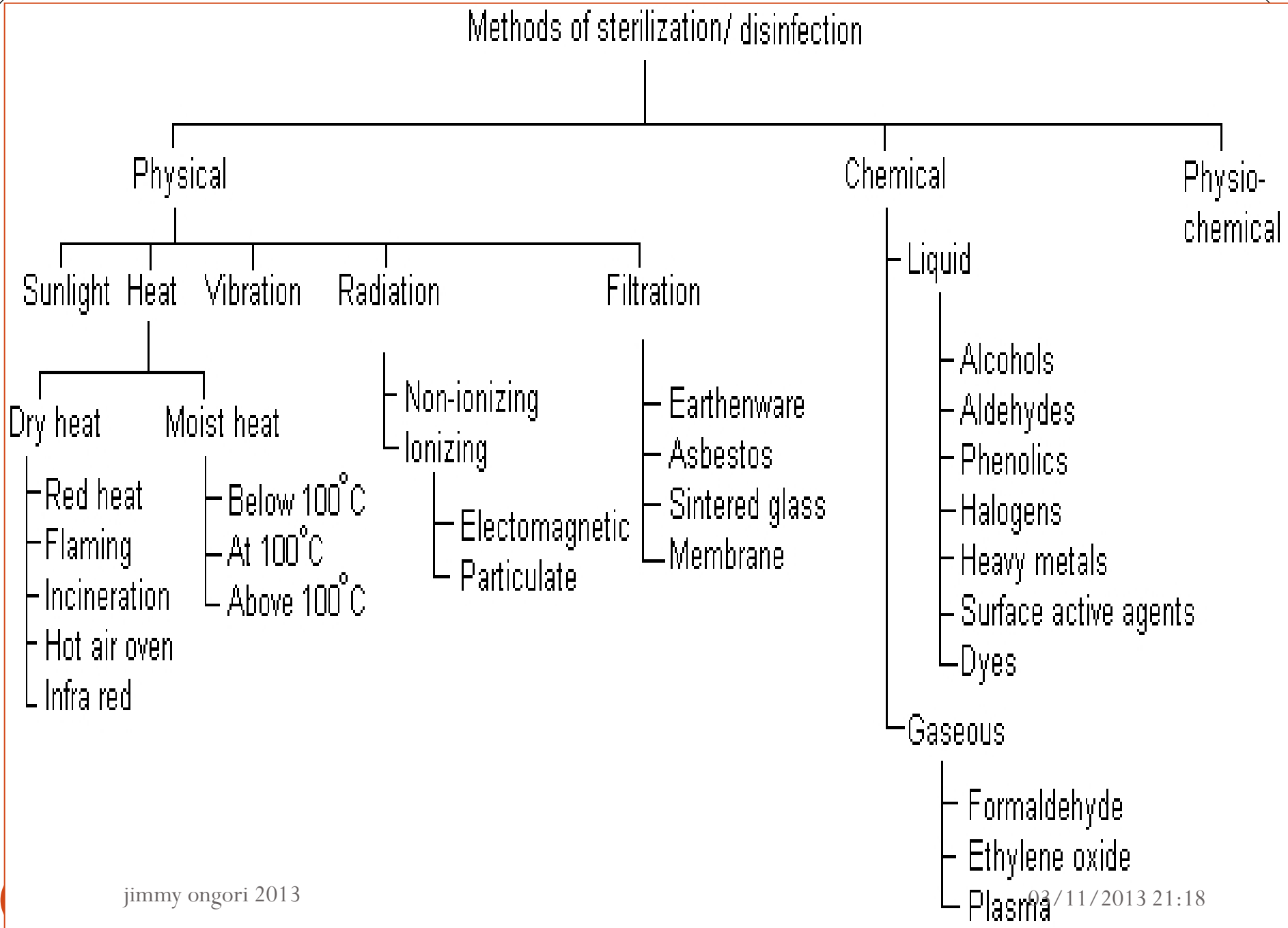
The Ideal Disinfectant

- Resistant to inactviation
- Broadly active (killing pathogens)
- Not poisonous (or otherwise harmful)
- Penetrating (to pathogens)
- Not damaging to non-living materials
- Stable
- Easy to work with
- Otherwise not unpleasant

Disinfectant Performance…

- Is dependent on Disinfectant concentrations
- Is dependent on length (time) of administration
- Is dependent on temperature during administration

  (usual chemical reaction 2x increase in rate with each 10°C increase in temperature)
- Microbe type (e.g., mycobacteria, spores, and certain viruses can be very resistant to disinfection—in general vegetative cells in log phase are easiest to kill)
- Substrate effects (e.g., high organic content interferes with disinfection—stainless steel bench easier to disinfect than wood)
- It is easier (and faster) to kill fewer microbes than many microbes


Methods of sterilization/ disinfection
Physical
Chemical
Physio-chemical
Sunlight
Heat
Vibration
Radiation
Filtration
Dry heat
Moist heat
Red heat
Flaming
Incineration
Hot air oven
Infra red
Below 100°C
At 100°C
Above 100°C
Non-ionizing
Ionizing
Electomagnetic
Particulate
Earthenware
Asbestos
Sintered glass
Membrane
Liquid
Alcohols
Aldehydes
Phenolics
Halogens
Heavy metals
Surface active agents
Dyes
Gaseous
Formaldehyde
Ethylene oxide
Plasma

PHYSICAL METHODS

1. Sunlight
2. Heat
   a) Dry heat – red heat, flaming heat, incineration, hot air oven, infra red heat
   b) Moist heat – at 100^0 C, $>100^0$ C, $< 100^0$ C
3. Vibration
4. Radiation – non-ionizing and ionizing
5. Filtration

PHYSICAL METHODS OF STERILIZATION

1. **Sunlight**

- The microbicidal activity of sunlight is mainly due to the presence of ultra violet rays in it
- It is responsible for spontaneous sterilization in natural conditions - due to combination of ultraviolet rays and heat.

PHYSICAL METHODS OF STERILIZATION

2. Heat

- Heat is considered to be most reliable method of sterilization of articles that can withstand heat.
- Heat acts by oxidative effects as well as denaturation and coagulation of proteins. Those articles that cannot withstand high temperatures can still be sterilized at lower temperature by prolonging the duration of exposure.

PHYSICAL METHODS OF STERILIZATION

Factors affecting sterilization by heat are:

- Nature of heat: Moist heat is more effective than dry heat
- Temperature and time: are inversely proportional. As temperature increases the time taken decreases.
- Number of microorganisms: More the number of microorganisms, higher the temperature or longer the duration required.
- Nature of microorganism: Depends on species and strain
- Spores are highly resistant to heat.

a) DRY HEAT:

i. **Red heat:** bacteriological loops, tips of forceps etc sterilized by holding them in Bunsen flame till they become red hot. Limited to articles that can be heated to redness in flame.

ii. **Flaming:** This is a method of passing the article over a Bunsen flame, but not heating it to redness eg scalpels, mouth of test tubes, glass slides and cover slips are passed through the flame a few times.

iii. **Incineration:** destroying contaminated material by burning in incinerator eg soiled dressings, pathological material and bedding etc . This technique results in the loss of the article, hence suitable only for those articles that have to be disposed.

iv. Hot air oven:

- This method was introduced by Louis Pasteur. Articles to be sterilized are exposed to high temp' (160^{o} C) for one hour in an electrically heated oven. Even distribution of heat throughout the chamber achieved by a fan.
- Articles sterilized: Metallic instruments (forceps, scalpels, scissors), glasswares (petri-dishes, pipettes, flasks, all-glass syringes), swabs, and some pharmaceutical products.
- Increasing temperature by 10 degrees shortens the sterilizing time by 50 percent.
- This is the only method of sterilizing oils and powders.

v. Infra red rays

- Infrared rays bring about sterilization by generation of heat.
- Articles to be sterilized are placed in a moving conveyor belt and passed through a tunnel that is heated by infrared radiators to a temperature of 180^{o}C for 7.5 minutes.
- Articles sterilized include metallic instruments and glassware.

b) MOIST HEAT

- Moist heat acts by coagulation and denaturation of proteins.

At temperature below 100°C:

i. Pasteurization:

- This process was originally used by Louis Pasteur.
- Currently this procedure is used in food and dairy industry. There are two methods of pasteurization, the holder method (heated at 63°C for 30 minutes) and flash method (heated at 72°C for 15 seconds) followed by quickly cooling to 13°C.

PHYSICAL METHODS OF STERILIZATION

- Other pasteurization methods include Ultra-High Temperature (UHT), 140°C for 15 sec and 149°C for 0.5 sec. This method is suitable to destroy most milk borne pathogens like Salmonella, Mycobacteria, Streptococci, Staphylococci and Brucella, however Coxiella may survive pasteurization. Others;

ii. **Vaccine bath**

iii. **Serum bath**

iv. **Inspissation**

- **At temperature 100°C:**

i) Boiling

- Boiling water (100°C) kills most vegetative bacteria and viruses immediately.
- Certain bacterial toxins such as Staphylococcal enterotoxin are heat resistant.
- Some bacterial spores are resistant to boiling and survive; hence this is not a substitute for sterilization.
- When absolute sterility is not required, certain metal articles and glasswares can be disinfected by placing them in boiling water for 10-20 minutes.

ii)

ii) Steam at 100°C

- **Free steam at 100°C**, An autoclave can be used. A steamer is a metal cabinet with perforated trays to hold articles.
- The bottom of steamer is filled with water and heated, steam generated sterilizes the articles when exposed for a period of 90 minutes. Media eg DCA and selenite broth sterilized by steaming.
- Sugar and gelatin in medium may get decomposed on autoclaving, hence they are exposed to free steaming for 20 minutes for 3 successive days. This process is known as **tyndallisation** (after John Tyndall) or fractional sterilization or intermittent sterilization. The vegetative bacteria are killed in the first exposure and the spores that germinate by next day are killed in subsequent days. The success of process depends on the germination of spores.

At temperature above 100°C:

Autoclave:

- Sterilization can be effectively achieved at a temperature above 100°C using an autoclave. Water boils at 100°C at atmospheric pressure, but if pressure is raised, the temperature at which the water boils also increases.
- In an autoclave the water is boiled in a closed chamber. As the pressure rises, the boiling point of water also raises.
- At a pressure of 15 lbs inside the autoclave, the temperature is said to be 121°C. Exposure of articles to this temperature for 15 minutes sterilizes them.

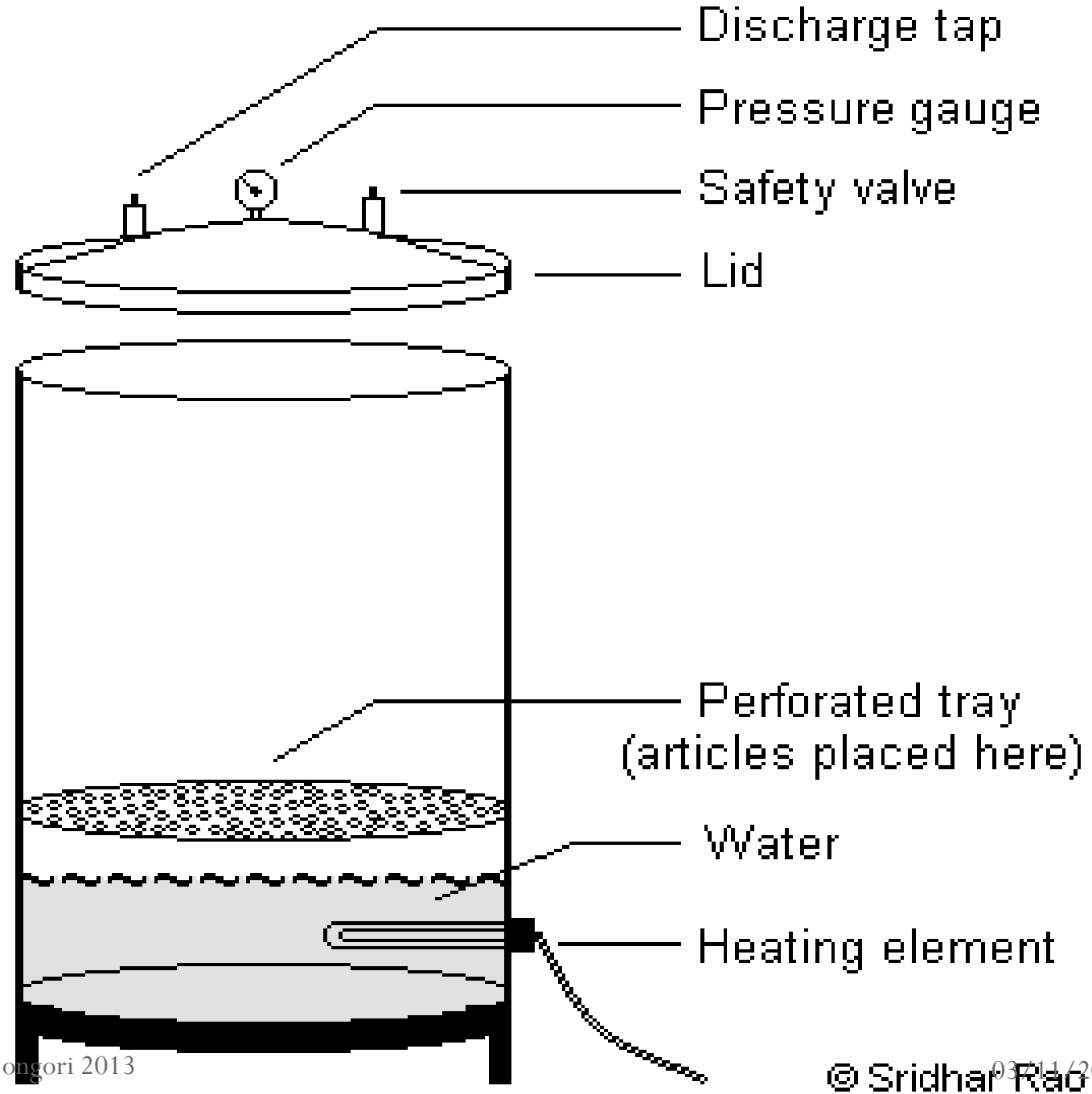
- To destroy the infective agents associated with spongiform encephalopathies (prions), higher temperatures or longer times are used; 135°C or 121°C for at least one hour are recommended.

Advantages of steam: It has more penetrative power than dry air, it moistens the spores (moisture is essential for coagulation of proteins

Articles sterilized: Culture media, dressings, certain equipment, linen etc.

Disadvantages: Drenching and wetting of articles may occur, trapped air may reduce the efficacy, takes long time to cool

AUTOCLAVE


Discharge tap
Pressure gauge
Safety valve
Lid
Perforated tray
(articles placed here)
Water
Heating element
© Sridhar Rao

RADIATION

- Two types of radiation are used, ionizing and non-ionizing.
- Non-ionizing rays are low energy rays with poor penetrative power while ionizing rays are high-energy rays with good penetrative power. Since radiation does not generate heat, it is termed "cold sterilization".

i) Non-ionizing rays:

- Rays of wavelength longer than the visible light are non-ionizing.
- UV rays are generated using a high-pressure mercury vapor lamp.
- UV rays induce formation of thymine-thymine dimers, which inhibits DNA replication.

RADIATION

- UV readily induces mutations in cells irradiated with a non-lethal dose. Microorganisms such as bacteria, viruses, yeast, etc. that are exposed to the effective UV radiation are inactivated within seconds.
- UV rays don't kill spores
- UV rays are used to disinfect hospital wards, operation theatres, virus laboratories, corridors, etc. Disadvantages - harmful to skin and eyes.

Ionizing Rays

- Used to sterilize articles like syringes, gloves, dressing packs, foods and pharmaceuticals, petri dishes, antibiotics, vitamins, hormones, glasswares and fabrics.
- Sterilization is accomplished in few seconds.

FILTRATION

- Filtration does not kill microbes, it separates them out.
- Membrane filters with pore sizes between 0.2-0.45 µm are commonly used to remove particles from solutions that can't be autoclaved.
- Used to remove microbes from heat labile liquids such as serum, antibiotic solutions, sugar solutions, urea solution.
- Filtration is aided by using either positive or negative pressure using vacuum pumps.

Types of filters

1. **Earthenware filters**: These filters are made up of diatomaceous earth or porcelain.
2. **Asbestos filters**
3. **Sintered glass filters:** These are made from finely ground glass that are fused sufficiently to make small particles adhere to each other.
4. **Membrane filters**
5. **Air Filters:** They are usually used in biological safety cabinets.

CHEMICAL METHODS

- Disinfectants are those chemicals that destroy pathogenic bacteria from inanimate surfaces. Some chemical have very narrow spectrum of activity and some have very wide. Those chemicals that can sterilize are called chemisterilants. Those chemicals that can be safely applied over skin and mucus membranes are called antiseptics.

Chemical Antimicrobials

Agent	Mechanisms of Action	Comments
Surfactants	Membrane Disruption; increased penetration	Soaps; detergents
Quats (cationic detergent)	Denature proteins; Disrupts lipids	Antiseptic - benzalconium chloride, Cepacol; Disinfectant
Organic acids and bases	High/low pH	Mold and Fungi inhibitors; e.g., benzoate of soda
Heavy Metals	Denature protein	Antiseptic & Disinfectant; Silver Nitrate
Halogens	Oxidizing agent Disrupts cell membrane	Antiseptic - Iodine (Betadine) Disinfectant - Chlorine (Chlorox)
Alcohols	Denatures proteins; Disrupts lipids	Antiseptic & Disinfectant Ethanol and isopropyl
Phenolics	Disrupts cell membrane	Disinfectant Irritating odor
Aldehydes	Denature proteins	Gluteraldehyde - disinfectant (Cidex); Formaldehyde - disinfectant
Ethylene Oxide	Denaturing proteins	Used in a closed chamber to sterilize
Oxidizing agents	Denature proteins	Hydrogen peroxide – antiseptic; Hydrogen peroxide – disinfectan; Benzoyl peroxide – antiseptic

Chemical Methods of Microbial Control

Types of Disinfectants

1. Phenols and Phenolics:

- **Phenol** (carbolic acid) was first used by Lister as a disinfectant.
  - Rarely used today because it is a skin irritant and has strong odor.
- **Phenolics** are chemical derivatives of phenol, Destroy plasma membranes and denature proteins.
  - **Cresols**: eg **Lysol**
  - **Biphenols**

2. Halogens: Effective alone or in compounds.

A. Iodine:

- **Tincture of iodine** (alcohol solution) was one of first antiseptics used, denatures proteins.
- Stains skin and clothes, somewhat irritating.
- **Iodophors**: Compounds with iodine that take several minutes to act. Used as skin antiseptic in surgery. Not effective against bacterial endospores eg; Betadine

B. Chlorine:

- When mixed in water forms **hypochlorous acid**, Used to disinfect drinking water, pools, and sewage.
- **Sodium hypochlorite -** active ingredient of bleach.
- **Chloramines**: Consist of chlorine and ammonia. Less effective as germicides.

3. Alcohols:

- Kill bacteria, fungi, but not endospores or naked viruses.
- Act by denaturing proteins and disrupting cell membranes. Evaporate, leaving no residue.
- Used to mechanically wipe microbes off skin before injections or blood drawing.
- Not good for open wounds, because cause proteins to coagulate.
  - **Ethanol**: Optimum concentration is 70%.
  - **Isopropanol**: Better disinfectant than ethanol. Also cheaper and less volatile.

4. Heavy Metals:

- Include copper, selenium, mercury, silver, and zinc.

A. Silver: 1% silver nitrate used to protect infants against gonorrheal eye infections until recently.

B. Copper; Copper sulfate is used to kill algae in pools and fish tanks.

C. Selenium: Kills fungi and their spores. Used for fungal infections. Also used in dandruff shampoos.

E. Zinc: Zinc chloride is used in mouthwashes.

- Zinc oxide is used as antifungal agent in paints.

5. Quaternary Ammonium Compounds:

- Widely used surface active agents.
- Effective against gram +VE bacteria, less effective against gram -VE bacteria. Also destroy fungi, amoebas, and enveloped viruses.
- **Zephiran**, Cepacol
- **Advantages**: Strong antimicrobial action, colorless, odorless, tasteless, stable, and nontoxic.
- **Diasadvantages**: Form foam. Organic matter interferes with effectiveness. Neutralized by soaps and anionic detergents.

6. Aldehydes:

- Include some of the most effective antimicrobials.
- Inactivate proteins by forming covalent crosslinks with several functional groups.

A. Formaldehyde gas:

- Excellent disinfectant.
- Commonly used as **formalin**
- Formalin is used extensively to preserve biological specimens and inactivate viruses and bacteria in vaccines.
- Irritates mucous membranes, strong odor.
- Also used in mortuaries for embalming.

B. Glutaraldehyde:

- Less irritating and more effective than formaldehyde.
- One of the few chemical disinfectants that is a **sterilizing agent**.
- A 2% solution of glutaraldehyde **(Cidex)** is:
  - Bactericidal, tuberculocidal, and viricidal in 10 minutes.
  - Sporicidal in 3 to 10 hours.
- Commonly used to disinfect hospital instruments.
- Also used in mortuaries for embalming.

7. Gaseous Sterilizers:

- Chemicals that sterilize in a chamber similar to an autoclave.

A. Ethylene Oxide:

- Kills all microbes and endospores, but requires exposure of 4 to 18 hours.
- Toxic and explosive in pure form.
- Highly penetrating.
- Many hospitals have ethylene oxide chambers to sterilize mattresses and large equipment.

8. Peroxygens (Oxidizing Agents):

- Oxidize cellular components of treated microbes.
- Disrupt membranes and proteins.

A. Ozone:

- Highly reactive form of oxygen.
- Used along with chlorine to disinfect water.
- Helps neutralize unpleasant tastes and odors.
- More effective killing agent than chlorine, but less stable and more expensive.
- Made by exposing oxygen to electricity or UV light.

B. Hydrogen Peroxide:

- Used as an antiseptic.
- Not good for open wounds because quickly broken down by catalase present in human cells.
- Effective in disinfection of inanimate objects.
- Sporicidal at higher temperatures.

C. Benzoyl Peroxide:

- Used in acne medications.

D. Peracetic Acid:

- One of the most effective liquid sporicides available.
- Kills bacteria and fungi in less than 5 minutes.
- Kills endospores and viruses within 30 minutes.
- Used widely in disinfection of food and medical instruments because it does not leave toxic residues.

BACTERIA

- The **bacteria** (*singular*: **bacterium**) are a large group of unicellular microorganisms. Typically a few micrometres in length, bacteria have a wide range of shapes, ranging from spheres to rods and spirals.
- Bacteria are ubiquitous in every habitat on Earth, growing in soil, acidic hot springs, radioactive waste, water, and deep in the Earth's crust, as well as in organic matter and the live bodies of plants and animals.
- There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a millilitre of fresh water
- Bacteria are vital in recycling nutrients, with many steps in nutrient cycles depending on these organisms, such as the fixation of nitrogen
- The study of bacteria is known as **bacteriology**, a branch of microbiology.

- There are about ten times as many bacterial cells in the human flora of bacteria as there are human cells in the body, with large numbers of bacteria on the skin and as gut flora.
- Majority of the bacteria in the body are rendered harmless by the protective effects of the immune system, and a few are beneficial. A few species of bacteria are pathogenic and cause infectious diseases.
- Bacteria are important in sewage treatment, the production of cheese and yoghurt through fermentation, as well as in biotechnology, and the manufacture of antibiotics and other chemicals.

- Once regarded as plants, bacteria are now classified as prokaryotes. Unlike cells of animals and other eukaryotes, bacterial cells do not contain a nucleus and rarely harbour membrane-bound organelles.
- Although the term *bacteria* traditionally included all prokaryotes, the scientific classification changed after the discovery in the 1990s that prokaryotes consist of two very different groups of organisms that evolved independently from an ancient common ancestor.
- These are called **Bacteria** and **Archaea**.
- Bacteria were first observed by Antonie van Leeuwenhoek in 1676, using a single-lens microscope of his own design. He called them "animalcules"

- The name ***bacterium*** was introduced much later, by Christian Gottfried Ehrenberg in 1838.
- Louis Pasteur demonstrated in 1859 that the fermentation process is caused by the growth of microorganisms, and that this growth is not due to spontaneous generation. (Yeasts and molds, commonly associated with fermentation, are fungi.)
- Along with Robert Koch, Pasteur was an early advocate of the germ theory of disease. Robert Koch was a pioneer in medical microbiology and worked on cholera, anthrax and tuberculosis. In his research into tuberculosis, Koch finally proved the germ theory. In *Koch's postulates*, he set out criteria to test if an organism is the cause of a disease these postulates are still used today.

PROKARYOTES AND EUKARYOTES

- "True" bacteria (which include all bacteria that infect man) are members of one kingdom (the eubacteria).
- A group of organisms often found in extreme environments form a second kingdom (archaebacteria, *Archaea*).
- Morphologically, the two kingdoms of organisms appear similar, especially in the absence of a nucleus, and thus are classified together as prokaryotes.

PROKARYOTES AND EUKARYOTES

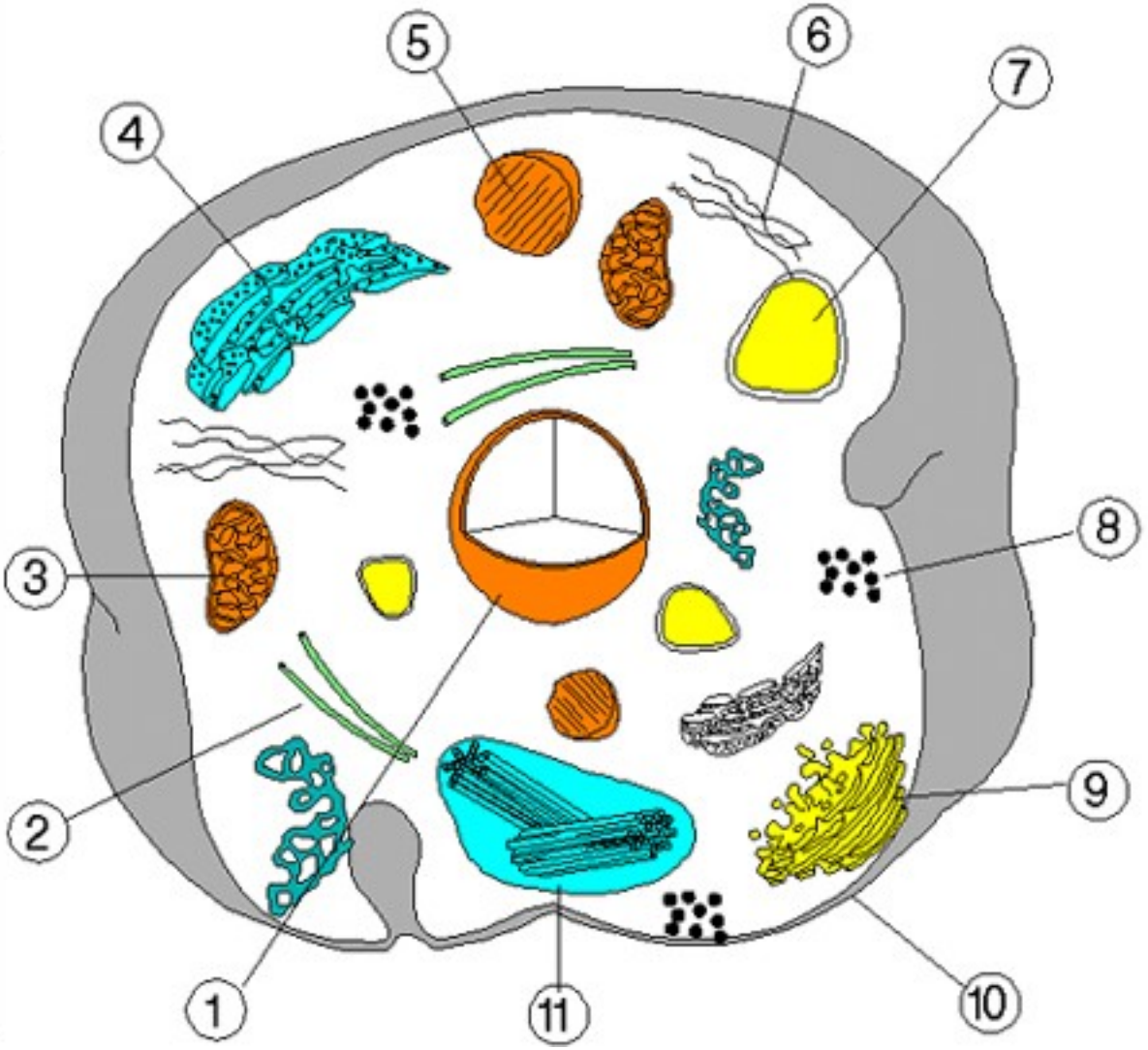
- However, they have major biochemical differences. Most archaea live in environments such as hot sulfur springs where they experience temperatures as high as 80^0 C and a pH 2. - called **thermoacidophiles**. Others live in methane-containing (methanogens) or high salt (extreme halophiles) environments.
- **Members of the *Archaea* are not human pathogens**

SUMMARY OF DIFFERENCES

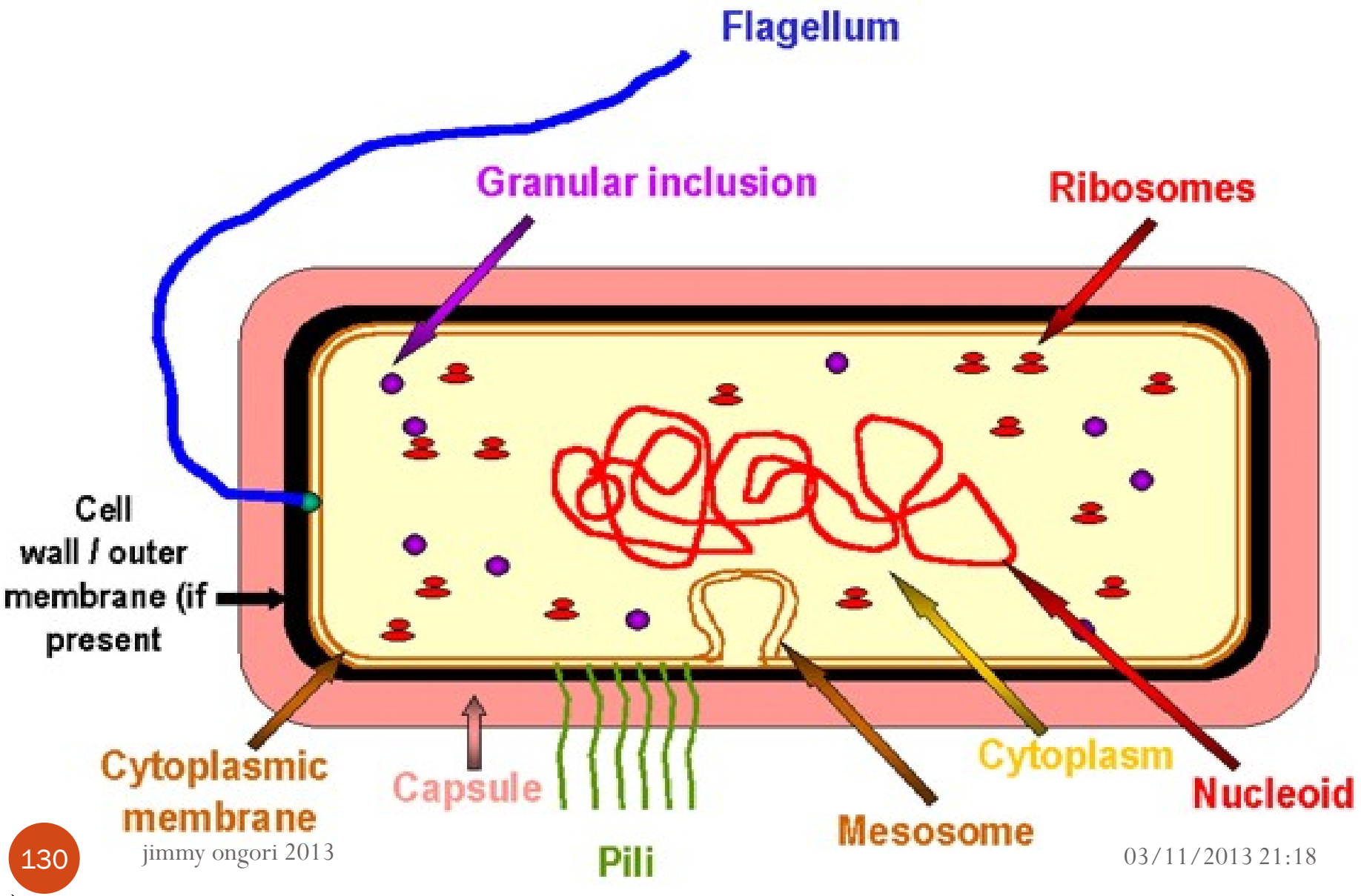
PROKARYOTIC CELLS	EUKARYOTIC CELLS
• Small cells (<5 μm)	• Larger cells (>10 μm)
• Always unicellular	• Often multicellular
• No nucleus or any membrane-bound organelles	• Always have nucleus and other membrane-bound organelles
• DNA is circular, without proteins	• DNA is linear and associated with proteins to form chromatin
• Ribosomes are small (70S)	• Ribosomes are large (80S)
• No cytoskeleton	• Always has a cytoskeleton
• Cell division is by binary fission	• Cell division is by mitosis or meiosis
• Reproduction is always asexual	• Reproduction is asexual or sexual

EUKARYOTIC CELL

The parts of a cell
1. Nucleus
2. Microtubule
3. Mitochondrion
4. Rough endoplasmic reticulum
5. Lysosome
6. Microfilaments
7. Vacuole
8. Ribosomes
9. Golgi complex
10. Cell membrane
11. Centrosome



THE PROTOTYPE BACTERIAL CELL



ASSIGNMENT

- **Draw a diagram of a Gram negative and Gram positive bacterial cell wall and briefly describe the differences**

BACTERIAL STRUCTURES

- Not all bacteria possess all of these components.

1. The cell envelope

- Bacteria can be divided into two groups on the basis of staining with the Gram stain; Gram positive bacteria remain stained by crystal violet on washing, Gram negative do not.
- All bacteria have a cell membrane where oxidative phosphorylation occurs (since there are no mitochondria). Outside the cell membrane is the cell wall which is rigid and protects the cell from osmotic lysis.

- In Gram +ve bacteria, the cell wall **peptidoglycan** layer is much thicker than in Gram -ve bacteria. Gram -ve bacteria have an additional outer membrane. The outer membrane is the major permeability barrier in Gram negative bacteria.
- The space between the inner and outer membranes is known as the periplasmic space. Gram -ve bacteria store degradative enzymes in the periplasmic space.
- Gram +ve bacteria lack a periplasmic space; instead they secrete exoenzymes and perform extracellular digestion.
- Digestion is needed since large molecules can not readily pass across the outer membrane (if present) or cell membrane.

THE BACTERIAL CELL WALL

- As in other organisms, the bacterial cell wall provides structural integrity to the cell. In prokaryotes, the primary function of the cell wall is to protect the cell from internal turgor pressure caused by the much higher concentrations of proteins and other molecules inside the cell compared to its external environment. The bacterial cell wall differs from that of all other organisms by the presence of **peptidoglycan**, which is located immediately outside of the cytoplasmic membrane.

THE BACTERIAL CELL WALL

- Peptidoglycan is responsible for the rigidity of the bacterial cell wall and for the determination of cell shape
- All bacterial cell walls (with a few exceptions e.g. *Mycoplasma*) contain peptidoglycan, not all cell walls have the same overall structures.
- There are two main types of bacterial cell walls, Gram positive and Gram negative, which are differentiated by their Gram staining characteristics.

TYPES OF BACTERIAL CELL ENVELOPES

Mycobacteria

- The Mycobacteria have a cell envelope which is not typical of Gram positives or Gram negatives.
- The mycobacterial cell envelope does not consist of the outer membrane characteristic of Gram negatives, but has a significant peptidoglycan-arabinogalactan-mycolic acid wall structure which provides an external permeability barrier.

The Gram positive cell wall

- The Gram positive cell wall has a very thick peptidoglycan layer, which is responsible for the retention of the crystal violet dyes during the Gram staining procedure.

The Gram negative cell wall

- It contains a **thin** peptidoglycan layer adjacent to the cytoplasmic membrane. This is responsible for the cell wall's inability to retain the crystal violet stain upon decolourisation with ethanol during Gram staining.
- In addition to the peptidoglycan layer, the Gram negative cell wall also contains an outer membrane composed by phospholipids and lipopolysaccharides whose chemical structure is often unique to specific bacterial strains (i.e. sub-species) and is responsible for many of the antigenic properties of these strains.

Plasmids

- These are extra-chromosomal DNA, usually present in multiple copies, that often code for pathogenesis factors and antibiotic resistance factors. Some forms are also involved in bacterial replication.

Flagella

- Some bacterial species are mobile and possess locomotory organelles – flagella. Those that do are able to taste their environment and respond to specific chemical foodstuffs or toxic materials and move towards or away from them (chemotaxis).
- Flagella are embedded in the cell membrane, extend through the cell envelope and project as a long strand. They move the cell by rotating with a propeller like action.

Pili (synonym: fimbriae)

- The types of pili varies both among and between species. Pili are hair-like projections of the cell.
- Some are involved in **sexual conjugation** and others allow **adhesion** to host epithelial surfaces in infection.

Capsules and slime layers

- Surround the outside of the cell envelope. When more defined, they are referred to as a capsule when less defined as a slime layer or glycocalyx.
- usually consist of polysaccharide; however, in certain bacilli they are composed of a polypeptide .
- Capsules of pathogenic bacteria **inhibit ingestion** and **killing by phagocytes**.

Endospores (spores)

(Read and make notes on spores)

- These are a dormant form of a bacterial cell produced by certain bacteria when starved; the actively growing form of the cell is referred to as vegetative. The spore is resistant to adverse conditions (including high temperatures and organic solvents).
- The spore cytoplasm is dehydrated and contains calcium dipicolinate which is involved in the heat resistance of the spore.
- Spores are commonly found in the genera *Bacillus* and *Clostridium*.

CELL MORPHOLOGY

- Bacteria display a wide diversity of shapes and sizes, called morphologies.
- Bacterial cells are about one tenth the size of eukaryotic cells and are typically 0.5–5.0 μm in length.
- A few species eg *Thiomargarita namibiensis* – are up to half a millimetre long and are visible to the unaided eye.
- Among the smallest bacteria are members of the genus Mycoplasma, which measure only 0.3 micrometres.
- Most bacterial species are; spherical, called **cocci** (Greek kókkos, grain/seed) or rod-shaped, called **bacilli** (Latin baculus, stick).
- Some rod-shaped bacteria, called **vibrio**, are slightly curved or comma-shaped; others, can be spiral-shaped, called **spirilla**, or tightly coiled, called **spirochaetes**.

CELL MORPHOLOGY Cont'

- This wide variety of shapes is determined by the bacterial cell wall and cytoskeleton
- Many bacterial species exist as single cells, others associate in characteristic patterns:
  - ***Neisseria*** form diploids (pairs)
  - ***Streptococcus*** form chains
  - ***Staphylococcus*** group together in "bunch of grapes" clusters.
  - Bacteria can also be elongated to form filaments, for example the Actinobacteria. Filamentous bacteria are often surrounded by a sheath that contains many individual cells.
  - Bacteria are classified by direct examination with the light microscope through its morphology and aggregation.

COCCI (singular - **coccus**)

- Are any bacteria whose overall shape is spherical or nearly spherical. Describing a bacterium as a coccus, or sphere, distinguishes it from bacillus, or rod. This is the first of many taxonomic traits for identifying and classifying a bacterium
- Basic forms;
  1. Pairs – *diplococci eg* ***Neisseria gonorrhoeae***
  2. Groups of 4 or 8 known as tetrads or *sarcina eg Micrococci*
  3. Bead-like chains, or *streptococci eg* ***Streptococcus pneumoniae***
  4. Grapelike clusters, or *staphylococci eg* ***Staphylococcus aureus***

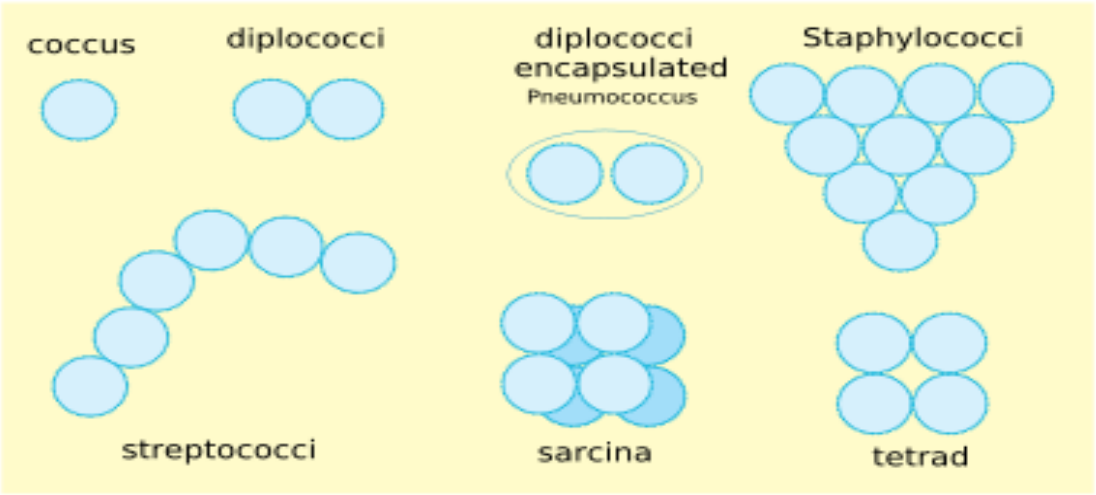
Bacillus

- Although *Bacillus* refers to the genus, the word **bacillus** may also be used to describe any rod-shaped bacterium, and in this sense, bacilli are found in many different taxonomic groups of bacteria.
- Bacilli are usually solitary, but can combine to form diplobacilli, streptobacilli, and palisades.

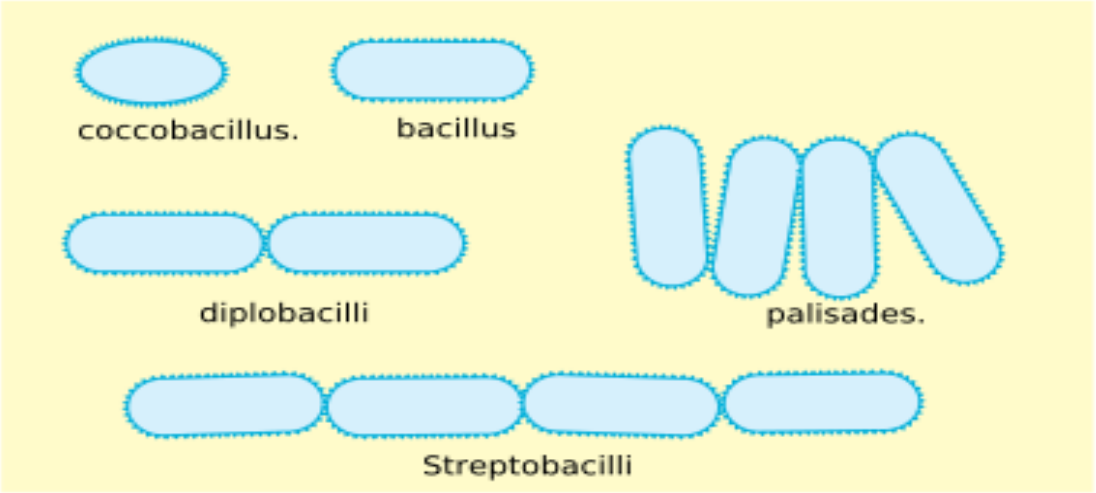
Coccobacillus

- A type of rod-shaped bacteria. The word *coccobacillus* reflects an intermediate shape between *coccus* and *bacillus*.
- Coccobacilli rods are so short and wide that they resemble cocci. ***Haemophilus influenzae*** and ***Chlamydia trachomatis*** are coccobacilli.

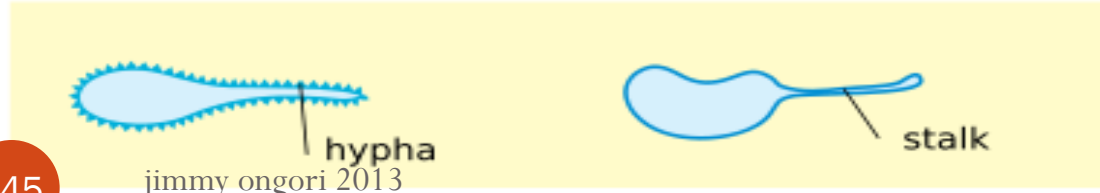
Cocci



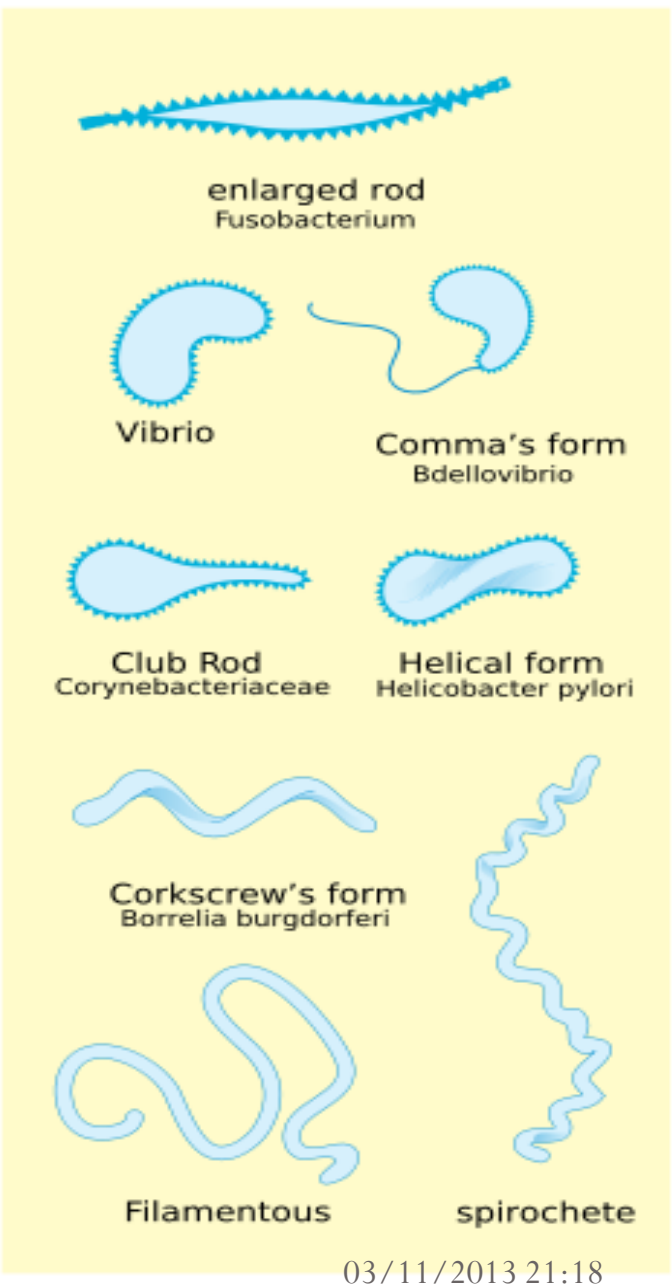
Bacilli



Budding and appendaged bacteria



Others



PATHOGENESIS OF BACTERIAL INFECTION

PATHOGENICITY TOXIGENICITY VIRULENCE

- The pathogenesis of bacterial infection includes the initiation of the infectious process and the mechanisms leading to the development of signs and symptoms of bacterial disease.
- The outcome of the interaction between bacteria and host is determined by characteristics that favour establishment of the bacteria within the host and their ability to damage the host as they are opposed by host defense mechanisms.
- Among the characterics of bacteria are;
  - **Adherence to host cells**
  - **Invasiveness**
  - **Toxigenity**
  - **Ability to evade the host´s immune system.**

Pathogenesis of bacterial infection

- Humans and animals have abundant normal microflora.
- Most bacteria do not produce disease but achieve a balance with the host that ensures the survival, growth, and propagation of both the bacteria and the host.
- Sometimes bacteria are pathogenic (e.g. *Salmonella typhi*) but infection remains **latent** or **subclinical** and the host is a **"carrier"** of the bacteria.
- Analysis of infection and disease through the application of principles such as **Koch´s postulates** leads to classification of bacteria as pathogenic or non-pathogenic.

- Some bacterial species are always considered to be pathogens, and their presence is abnormal. Eg *Mycobacterium tuberculosis* and *Yersinia pestis* (plague).
- Some sp. are part of the normal flora of humans but can cause disease. Eg *Escherichia coli* is part of the GIT flora of normal humans, but it is also a comon cause of urinary tract infection and traveller´s diarrhea,

The infectious process

- Infection indicates multiplication of M.O.s
- Prior to multiplication, bacteria must enter and establish themselves within the host.
- The most frequent portals of entry;
  - the respiratory (mouth and nose)
  - gastrointestinal
  - urogenital tracts
  - abnormal areas of mucous membranes and skin (e.g. cuts, burns) are also frequent sites of entry.

The infectious process

- Once in the body, bacteria must attach or adhere to host cells - usually epithelial cells.
- After the bacteria have established a primary site of infection, they multiply and spread.
- Infection can spread directly through tissues or via the lymphatic system to bloodstream.
- Bloodstream infection is known as **bacteremia**.
- Bacteremia allows bacteria to spread widely in the body and permits them to reach tissues suitable for their multiplication.

TERMINOLOGIES

- ***Infection*:**
  - Multiplication of an infectious agent within the body.
  - Multiplication of the bacteria that are part of normal flora is generally not considered an infection.
- ***Pathogenicity*:**
  - The ability of an infectious agent to cause disease.
- ***Virulence*:**
  - The degree or extent of pathogenicity
  - The quantitative ability of an agent to cause disease.
  - Virulence involves **invasiveness** and **toxigenicity**.

Virulence factors

- These are traits or features that allow or enhance the microorganism's ability to cause disease, include;
  i. adhesion organelles,
  ii. toxin production
  iii. evasion of the host's immune response
  iv. resistance to antibiotics
  v. ability to invade host tissues
  vi. enhanced intracellular survival and growth

- ***Toxigenicity*:**
  - ability of a microorganism to produce a toxin that contributes to the development of disease.
- ***Invasion*:**
  - The process whereby bacteria, parasites, fungi and viruses enter the host cells or tissues and spread in the body.
- ***Pathogen*:**
  - A microorganism capable of causing disease.
- ***Opportunistic pathogen*:**
  - An agent capable of causing disease only when the host´s resistance is impaired (e.g. the patient is immunocompromised).

Bacterial virulence factors

1. TOXINS

- Toxins produced by bacteria are generally classified into two groups:
  - Exotoxins
  - Endotoxins - Only found in gram negative bacteria

Endotoxins of gram-negative bacteria

- Endotoxins are toxic components of the bacterial cell envelope.
- The classical and most potent endotoxin is lipopolysaccharide derived from bacterial cell walls and are liberated when the bacteria lyse.
- The **pathophysiologic effects of LPS** are similar regardless of their bacterial origin
- Relatively stable; withstand heating at temperatures above 60°C for hours without loss of toxicity
- Usually produce fever in the host

Exotoxins

- Many gram-positive and gram-negative bacteria produce exotoxins of considerable medical importance - that modify, by enzymatic action, or destroy certain cellular structures.
- Examples - botulism, anthrax, cholera and diphtheria.
- Vaccines have been developed for some of the exotoxin-mediated diseases and continue to be important in the prevention of disease.
- These vaccines—called **toxoids**—are made from exotoxins, which are modified so that they are no longer toxic

1. Diphtheria toxin (*Corynebacterium diphtheriae*)

- It is a Gm +ve rod that can grow on the mucous membranes of the upper respiratory tract or in minor skin wounds. Some strains produce diphtheria toxin.

2. Tetanospasmin (toxin of *Clostridium tetani*)

- *C. tetani* is an anaerobic gram-positive rod
- It contaminates wounds, and the spores germinate in the anaerobic environment of the devitalized tissue. The vegetative forms of *Clostridium tetani* produce toxin tetanospasmin.
- Toxin reaches the CNS and acts by blocking release of an inhibitory mediator in motor neuron synapses.
- Extremely small amount of toxin can be lethal for humans.

3. Botulotoxin (toxin of *Clostridium botulinum*)

- *Clostridium botulinum* is found in soil or water and may grow in foods if the environment is appropriately anaerobic.
- It is the most potent toxin known – causes botulism
- It is heat-labile and is destroyed by sufficient heating.
- Toxin is absorbed from the GIT and carried to motor nerves, where it blocks the release of acetylcholine at synapses and neuromuscular junctions. Muscle contraction does not occur, and paralysis results.

4. Toxins of *Clostridium perfringens*

- Spores of *Clostridium perfringens* are introduced into the wounds by contamination with soil or faeces. In the presence of necrotic tissue, spores germinate and vegetative cells produce several different toxins.
- Many are necrotizing and hemolytic and favour the spread of **gangrene**

5. Streptococcal erythrogenic toxin

- Some strains of hemolytic streptococci produce a toxin that results in a rash, as in scarlet fewer.

6. Toxic shock syndrom toxin - 1 (TSST-1)

- Some *Staphylococcus aureus* strains growing on mucous membranes (e.g. on the vagina in association with menstruation), or in wounds, produce TSST-1.
- Toxic shock syndrome is characterized by shock, high fewer, and a diffuse red rash

7. Exotoxins associated with diarrheal diseases

- *Vibrio cholerae* toxin
- *Staphylococcus aureus* enterotoxin
- Other enterotoxins are produced; *Yersinia enterocolitica, Vibrio parahaemolyticus, Aeromonas species*

Enzymes

- **Many bacteria produce enzymes that play an important role in the infectious process;**

i. ***Collagenase:*** degrades collagen, the major protein of fibrous connective tissue, and promotes spread of infection in tissue.

ii. ***Coagulase*:** *Staphylococccus aureus* produce coagulase, which coagulates plasma. Coagulase contributes to the formation of fibrin walls around staphylococcal lesions, which helps them persist in tissues.

iii. ***Hyaluronidases*:** hydrolyze hyaluronic acid, a constituent of substance of connective tissue (e.g. staphylococci, streptococci and anaerobes) and aid in their spread through tissues.

Enzymes

iv. ***Streptokinase*:** many hemolytic streptococci produce streptokinase (fibrinolysin), a substance that activates a proteolytic enzyme of plasma. It dissolves coagulated plasma and aids in the spread of streptococci through tissues. ***Streptokinase is used in treatment of acute myocardial infarction to dissolve fibrin clots***.

v. ***Hemolysins and leukocidins*:** Many bacteria produce substances that are cytolysins - they dissolve red blood cells (hemolysins) or kill tissue cells or leukocytes (leukocidins).

Antiphagocytic factors

- Many bacterial pathogens are rapidly killed once they are ingested by polymorphonuclear cells or macrophages.
- Some pathogens evade phagocytosis or leukocyte microbidical mechanisms e.g. *Streptococcus pneumoniae* have polysaccharide capsules.

Adherence factors

- Once bacteria enter the body of the host, they must adhere to cells of a tissue surface. If they do not adhere, they would be swept away by mucus and other fluids
- Adherence is followed by development of microcolonies and subsequent complex steps in the pathogenesis of infection.

STAINING

Staining

- **Staining** is a technique used in microscopy to enhance contrast in the microscopic image.
- Stains and dyes are frequently used in biology and medicine to highlight structures in biological tissues for viewing, often with the aid of different microscopes.
- Stains may be used to define and examine bulk tissues (highlighting, muscle fibers or connective tissue), classifying different blood cells.

Staining

- In biochemistry it involves adding a class-specific (DNA, proteins, lipids, carbohydrates) dye to a substrate to qualify or quantify the presence of a specific compound
- Biological staining is also used to mark cells in flow cytometry, and in gel electrophoresis.
- In vivo staining is the process of dyeing living tissues
- By causing certain cells or structures to take on contrasting colours, their morphology or position within a cell can be distinguished

Staining

- In vitro staining involves colouring cells or structures that are no longer living. Certain stains are often combined to reveal more details and features than a single stain alone.
- A counter-stain is a stain that makes cells or structures more visible, when not completely visible with the principal stain.
- For example, crystal violet stains only Gram +ve bacteria during Gram staining. A safranin counterstain is applied which stains all cells, allowing the identification of Gram -ve bacteria .

GRAM STAINING

- The method is named after its inventor, *Hans Christan Gram* (1853–1938)
- Gram staining is a bacteriological laboratory technique used to differentiate bacterial species into two large groups (Gram +ve and Gram -ve) based on the physical properties of their cell walls
- In a modern molecular microbiology lab, most identification is done using genetic sequences and other molecular techniques, which are more specific than differential staining.

- The Gram stain is the most widely used staining procedure in bacteriology.
- It is called a **differential stain** since it differentiates between Gram+VE and Gram-VE negative bacteria.
- Bacteria that stain **purple** with the Gram staining procedure are termed **Gram +VE;** those that stain **pink** are said to be **Gram -VE**. The terms have nothing to do with electrical charge, but simply designate two distinct morphological groups of bacteria.

- **Gram-positive and Gram-negative bacteria stain differently because of fundamental differences in the structure of their cell walls.**
- **The bacterial cell wall serves to give the organism its size and shape as well as to prevent osmotic lysis.The material in the bacterial cell wall which confers rigidity is peptidoglycan.**

Principles Gram Staining

- Gram stains are performed on body fluid or biopsy
- Gram staining tests the bacterial cell wall's ability to retain ***crystal violet*** dye during solvent treatment.
- **Safranin** is added as a mordant to form the *crystal violet/safranin* complex in order to render the dye impossible to remove.
- **Ethyl-alcohol** solvent acts as a decolorizer and dissolves the lipid layer from gram-negative cells. This enhances leaching of the primary stain from the cells into the surrounding solvent.
- Ethyl-alcohol will dehydrate the thicker gram-positive cell walls, closing the pores as the cell wall shrinks.
- For this reason, the diffusion of the crystal violet-safranin staining is inhibited, so the bacteria remain stained.

STAINING MECHANISM

- Gram +ve bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of cell wall), which are stained purple by crystal violet
- Gram -ve bacteria have a thinner layer (10% of cell wall), which are stained pink by the counter-stain. There are four basic steps of the gram stain:

1. Applying a primary stain (crystal violet) to a heat-fixed (death by heat) smear
2. The addition of a trapping agent (gram's iodine)
3. Rapid decolorization with alcohol or acetone
4. *Counterstaining* with safranin.

PROCEDURE FOR G/STAIN

(1) Fix smear by heat.

(2) Cover with crystal violet for 30-60 seconds

(3) Wash with water. Do not blot.

(4) Cover with Gram's iodine for 30-60 seconds

(5) Wash with water. Do not blot.

(6) Decolorize for 10–30 seconds with gentle agitation in acetone-alcohol

(7) Wash with water. Do not blot.

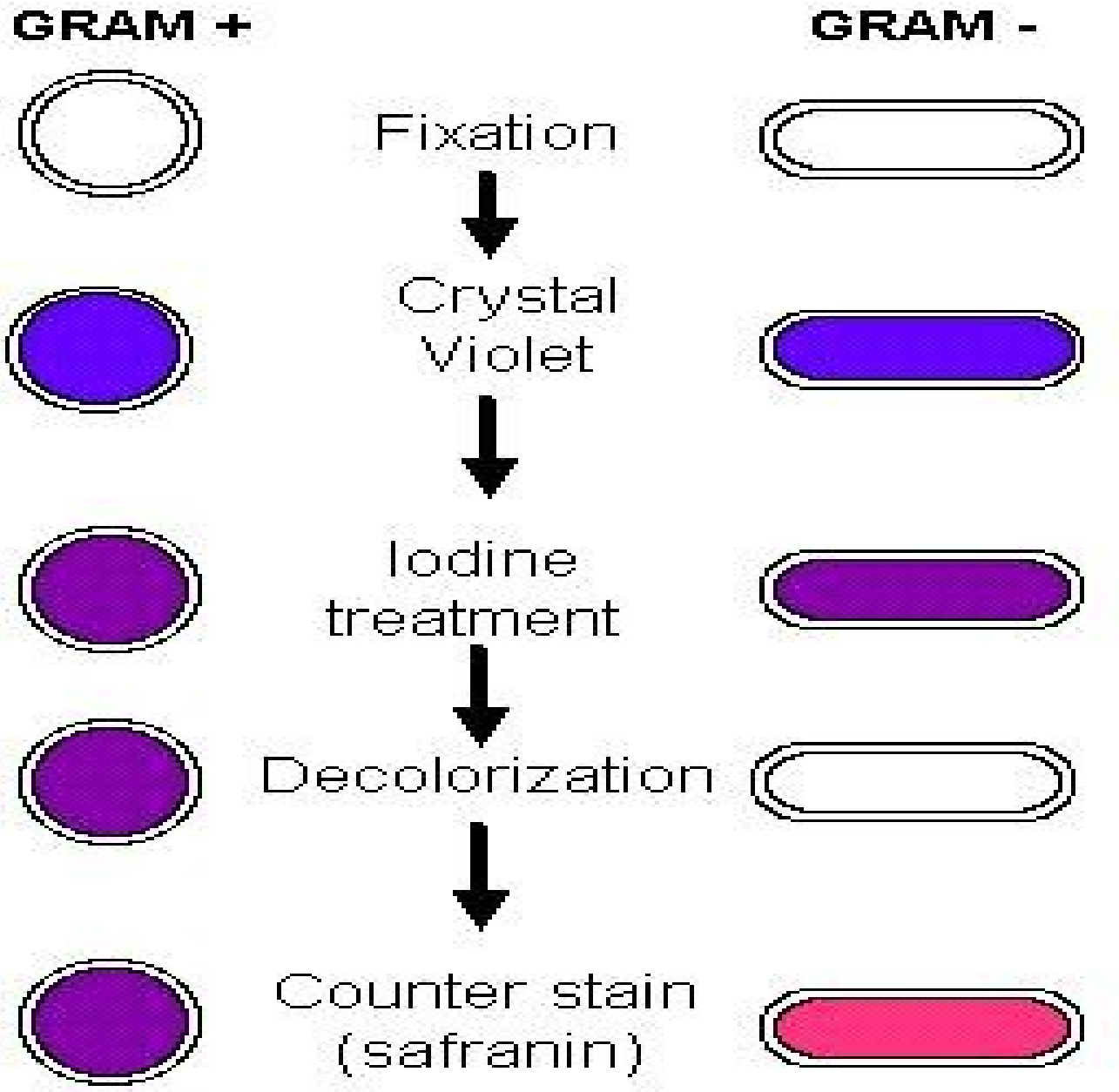
(8) Cover for 10–30 seconds with safranin (2.5% solution in 95% alcohol).

(9) Wash with water and let dry.

(10) Examine under oil immersion objective

- Gram-positive bacteria stain dark blue or violet
- Gram-negative organisms will appear red or pink because they are counterstaine

- Step 1. Staining with crystal violet.
- Step 2. Fixation with iodine stabilizes crystal violet staining. All bacteria remain purple or blue.
- Step 3. Extraction with alcohol or other solvent. Decolorizes some bacteria (Gram negative) and not others (Gram positive).
- Step 4. Counterstaining with safranin. Gram positive bacteria are already stained with crystal violet and remain purple. Gram negative bacteria are stained pink.


GRAM +
GRAM -
Fixation
Crystal Violet
Iodine treatment
Decolorization
Counter stain (safranin)

ZIEHL–NEELSEN STAIN

- The **Ziehl–Neelsen stain**, also known as the **acid-fast stain**, was first described by two German doctors; Franz **Ziehl** (1859 to 1926), and Friedrich **Neelsen** (1854 to 1898)
- It is a special bacteriological stain used to identify **acid-fast** organisms, mainly Mycobacteria.
- It is helpful in diagnosing *M. tuberculosis* since its lipid rich cell wall makes it resistant to Gram stain.
- The reagents used are Ziehl–Neelsen carbolfuchsin, acid alcohol and methylene blue. Acid-fast bacilli will be bright red after staining.

Acid-Fast Principles

- Primary stain penetrates cell wall
- Intense decolourization does not release primary stain from the cell wall of AFB
- Colour of AFB-based on primary stain
- Counterstain provides contrasting background

PROCEDURE

1. Heat fix the smear
2. Cover with carbol fuchsin stain
3. Heat the until vapour just begins to rise, do not overheat. Allow the heated stain to remain on the slide for 5 minutes
4. Wash off the stain with clean water
5. Cover the stain with 3% acid alcohol for 5 minutes or until the stain is fully decolorized ie pale pink

Procedure cont'

6. Wash with clean water
7. Cover the smear with methylene blue counter stain for 1 minute
8. Wash with clean water
9. Air dry on a rack
10. Examine using 100X oil immersion objective

THE COCCI

COCCI

- The cocci are one of the most commonly encountered bacteria (the other being bacilli)
- Cocci refers to the spherical shape
- Can exist as;
  - Single cells
  - Diplococcus eg *Neisseria*
  - Long chains – *Streptococcus, Enterococcus* and *Lactococcus*
  - Irregular clumps – *Staphyloccus*
  - Tetrads – genus *Micrococcus*
  - Cubical packets of 8 cells – genus *Sarcina*

STAPHYLOCCUS

- Are gram-positive spherical cells, usually arranged in grape-like irregular clusters.
- Some are members of the normal flora of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicaemia.
- Pathogenic staphylococci often haemolyse blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins.
- The most common type of food poisoning is caused by a heat-stable staphylococcal enterotoxin.

- Staphylococci rapidly develop resistance to many antimicrobial agents
- The genus *Staphylococcus* has at least 35 species.
- The three main species of clinical importance are ***S. aureus, S. epidermidis,*** and ***S. saprophyticus.***
- ***S aureus*** is a major pathogen for humans. Almost every person will have some type of *S aureus* infection during a lifetime, ranging in severity from food poisoning or minor skin infections to severe life-threatening infections.

General Characteristics

- Gram-positive cocci, nonmotile,
- Facultative anaerobes
- Cells occur in grapelike clusters
- Do not form spores but may have capsules
- Salt-tolerant: allows them to tolerate the salt present on human skin
- Tolerate desiccation: allows survival on environmental surfaces (fomites)

STAPHYLOCOCCUS AUREUS

- Many neonates, most children and adults become transiently colonized by *S. aureus.*
- The organism is carried in the nasopharynx, occasionally on their skin and clothing and more rarely in the vagina, in the rectum and or perineal area.
- One of the commoner causes of opportunistic infections in the hospital and community; including pneumonia, osteomyelitis, septic arthritis, bacteremia, endocarditis, abscesses/boils and other skin infections.

Virulence factors of *S. aureus*

Enzymes:

- Coagulase – coagulates plasma and blood; produced by 97% of human isolates; it is diagnostic
- Hyaluronidase – digests connective tissue
- Staphylokinase – digests blood clots
- DNase – digests DNA
- Lipases – digest oils; enhances colonization on skin
- Penicillinase – inactivates penicillin (very important in resistance to penicillins)

Virulence factors of *S. aureus*

Toxins:

- **Hemolysins** – lyse red blood cells
- **Leukocidin** – lyses neutrophils and macrophages
- **Enterotoxin** – induce gastrointestinal distress
- **Exfoliative toxin** – separates the epidermis from the dermis
- **Toxic shock syndrome toxin** (TSST) – induces fever, vomiting, shock, systemic organ damage

Epidemiology

- Present in most environments frequented by humans
- Readily isolated from fomites
- Carriage rate for healthy adults is 20-60%, mostly in anterior nares, skin, nasopharynx, intestine
- Predisposition to infection include: poor hygiene and nutrition, tissue injury, pre-existing primary infection, diabetes, immunodeficiency
- Increase in community acquired methicillin resistance - MRSA

Staphylococcal Disease

1. Localized cutaneous infections

- **Folliculitis** – superficial inflammation of hair follicle
- **Furuncle** – boil; inflammation of hair follicle or sebaceous gland progresses into abscess/pustule
- **Carbuncle** – larger and deeper lesion created by aggregation and interconnection of a cluster of furuncles
- **Impetigo** – bubble-like swellings that can break and peel away; most common in newborns
- **Abscess -** Predominant pathogen in about 50% of skin abscesses

2. Systemic Disease

i. **Toxic shock syndrome -** TSS toxin is absorbed into the blood and causes shock. Toxic shock syndrome is manifested by onset of high fever, vomiting, diarrhea, myalgias, rash, and hypotension with cardiac and renal failure in the most severe cases.

- Occurs within 5 days after the onset of menses in young women who use tampons, but can occur in children or in men with staphylococcal wound infections. TSS -associated *S aureus* can be found in the vagina, on tampons, in wounds or other localized infections, or in the throat but virtually never in the bloodstream.

- TSS -associated *S aureus* can be found in the vagina, on tampons, in wounds or other localized infections, or in the throat but virtually never in the bloodstrea

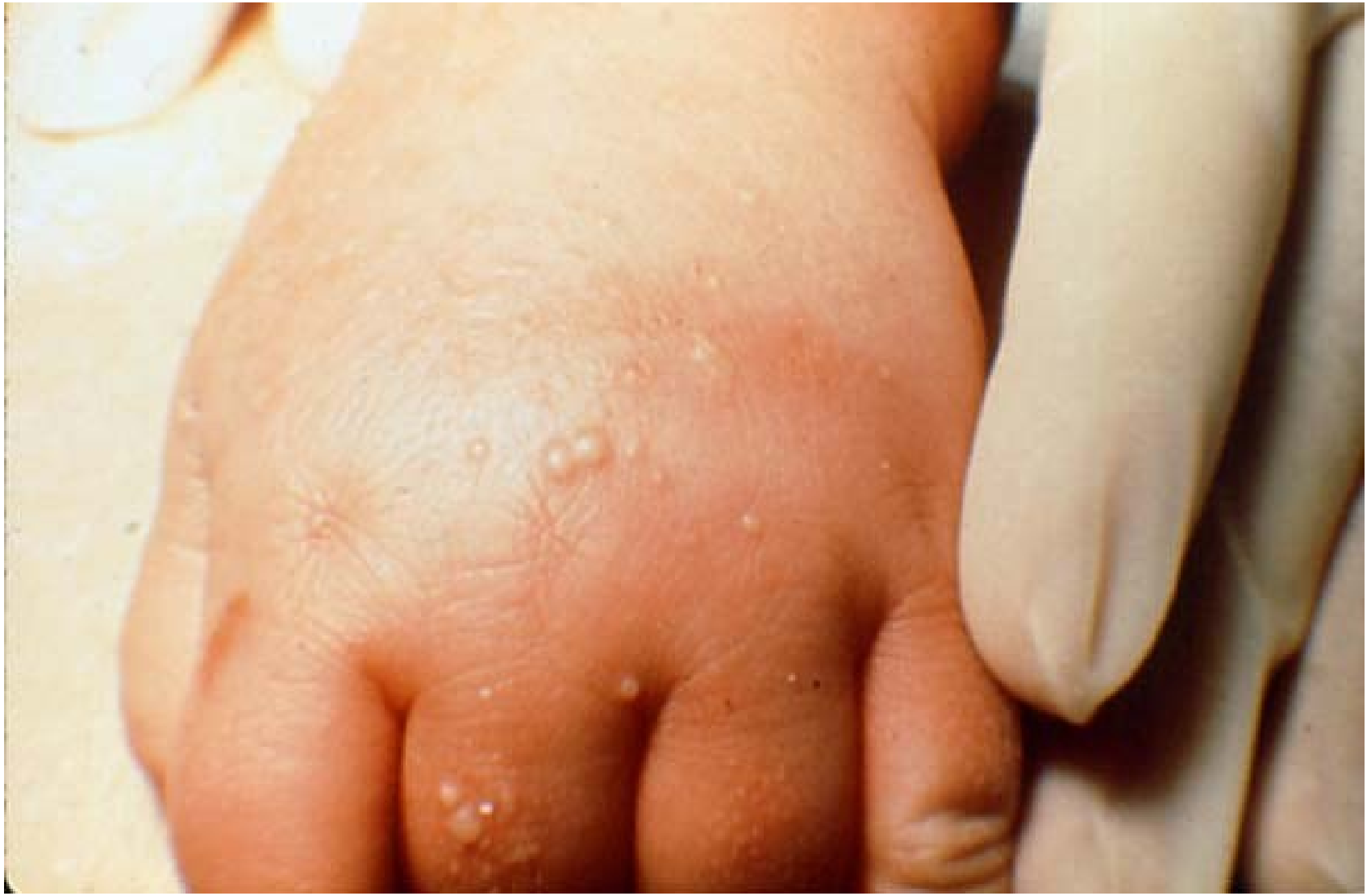
ii. **Bacteremia** - presence of bacteria in the blood

iii. **Endocarditi**s - occurs when bacteria attack the lining of the heart

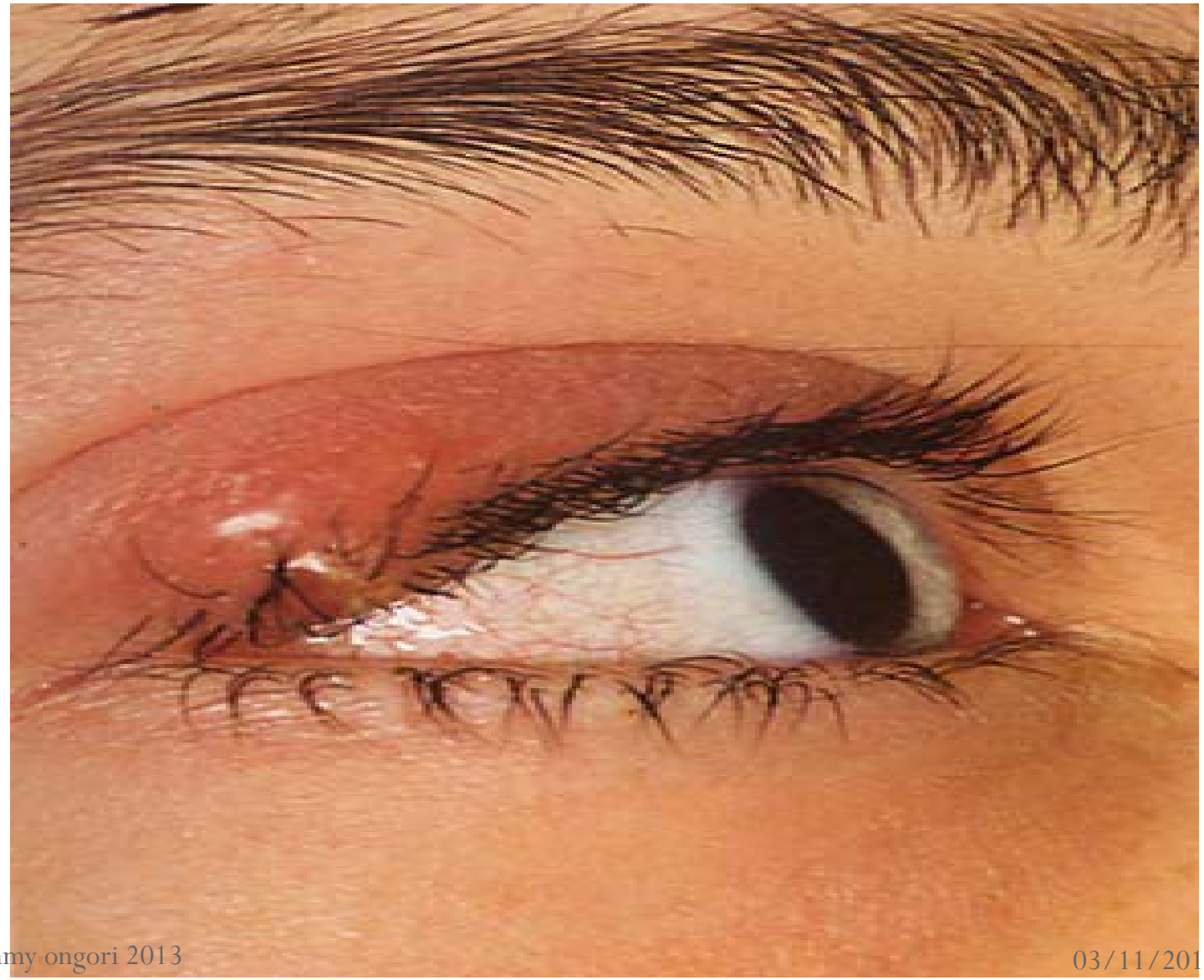
iv. **Pneumonia** - inflammation of the lungs

v. **Osteomyelitis** - inflammation of the bone marrow and the surrounding bone

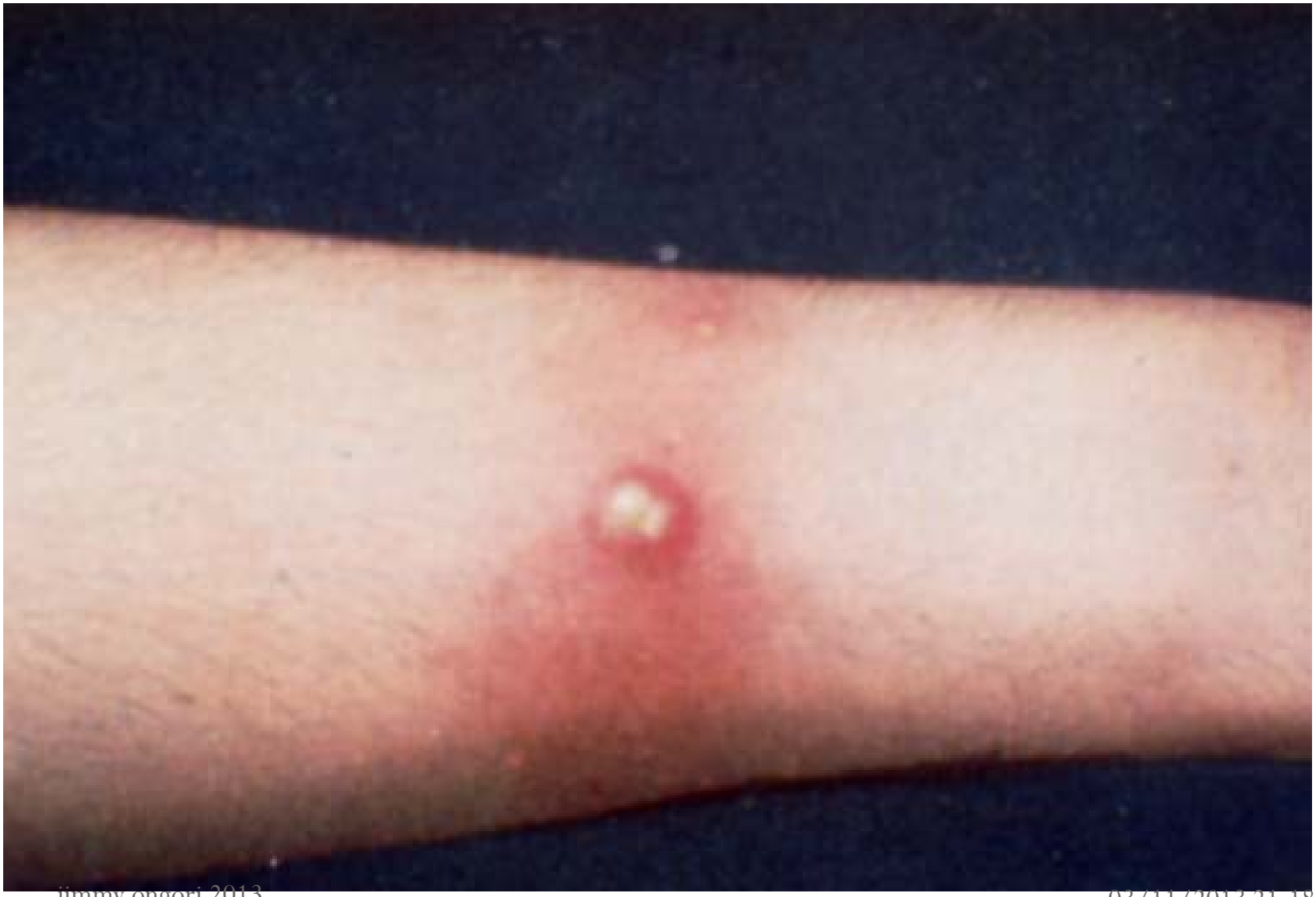
STAPHYLOCOCCAL FOLLICULITIS

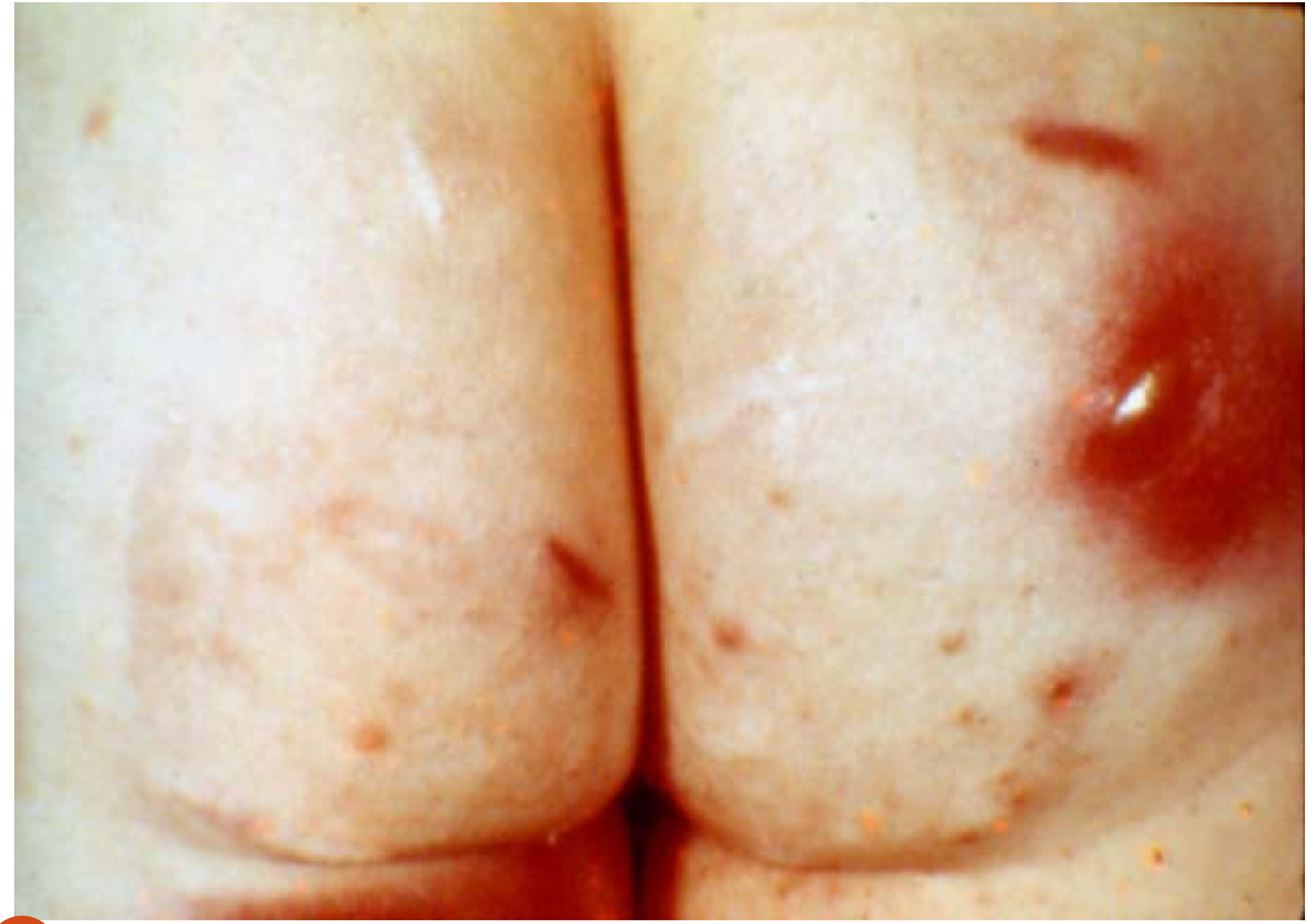


STYE

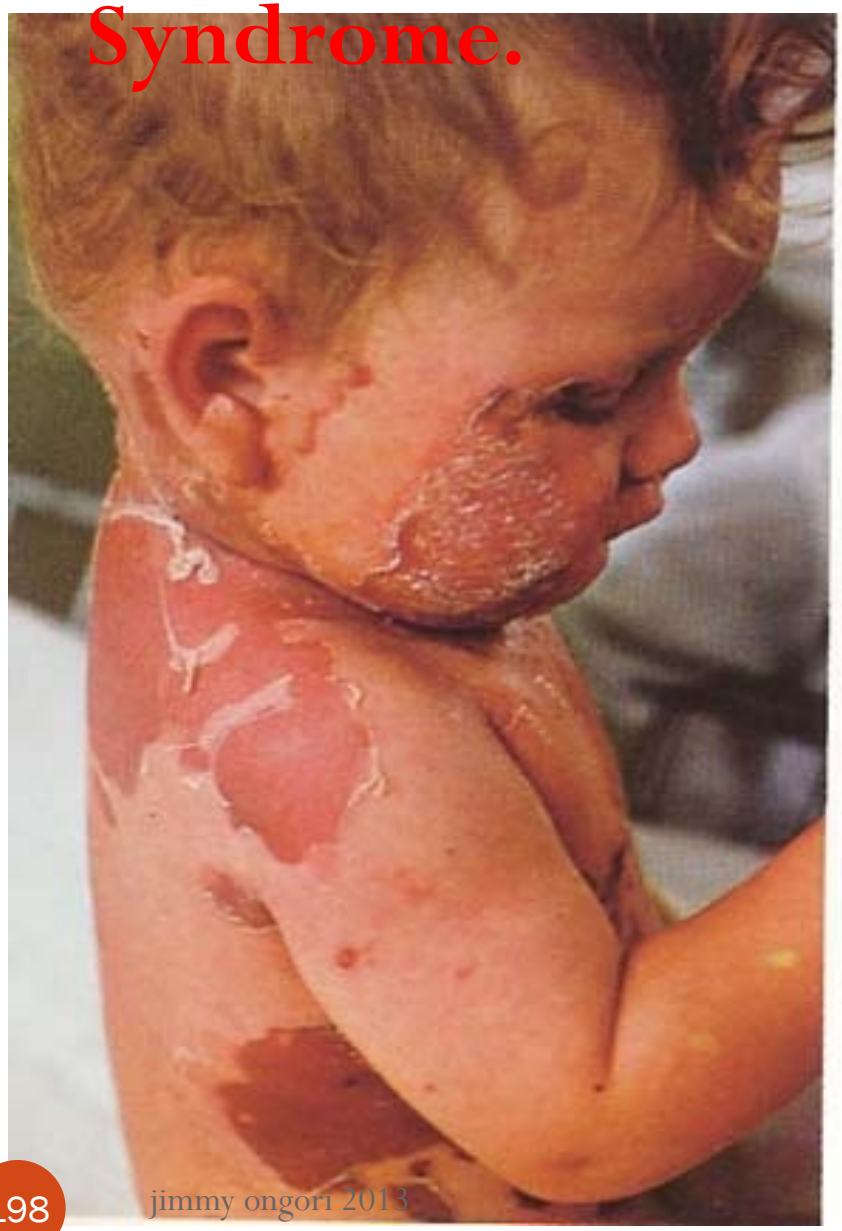


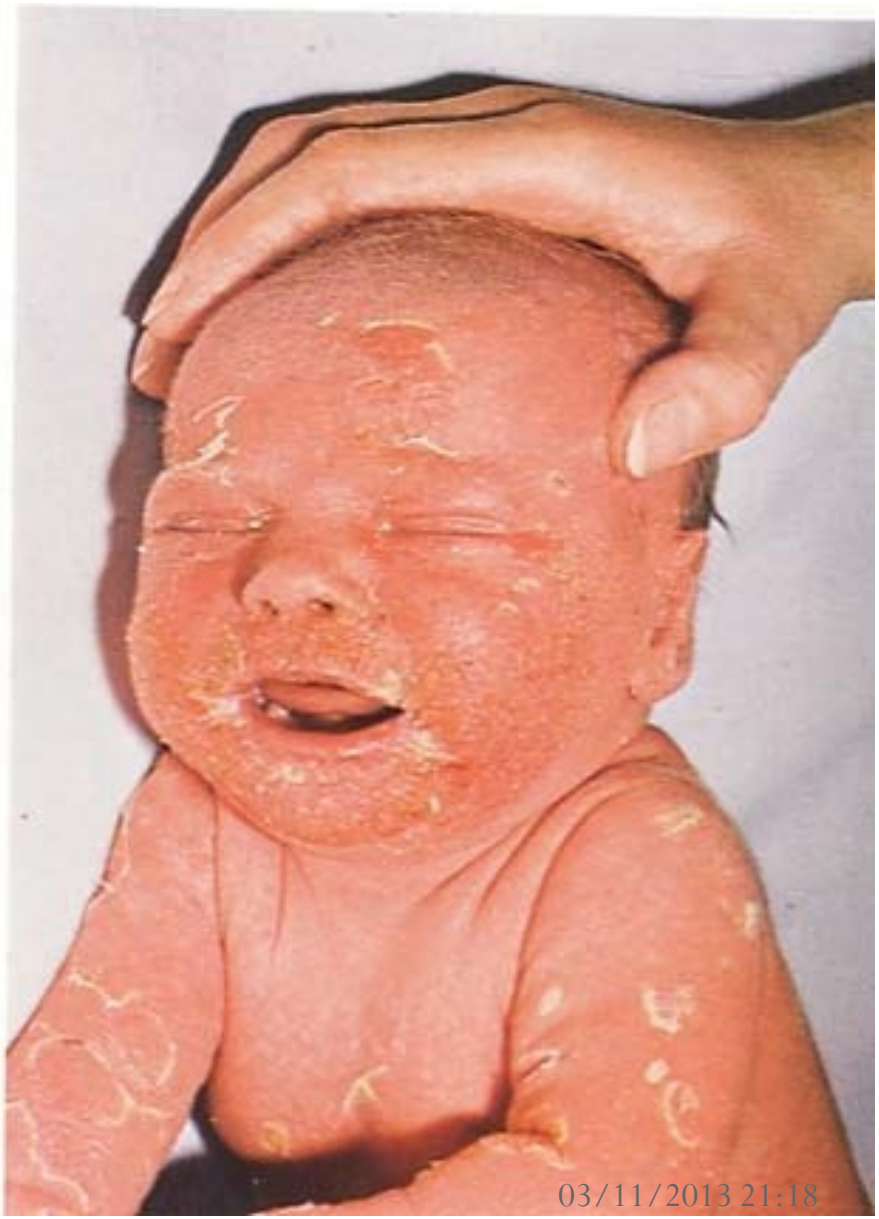
STAPHYLOCOCCAL FURUNCLE - " BOIL"





Staphylococcal Scalded Skin Syndrome.



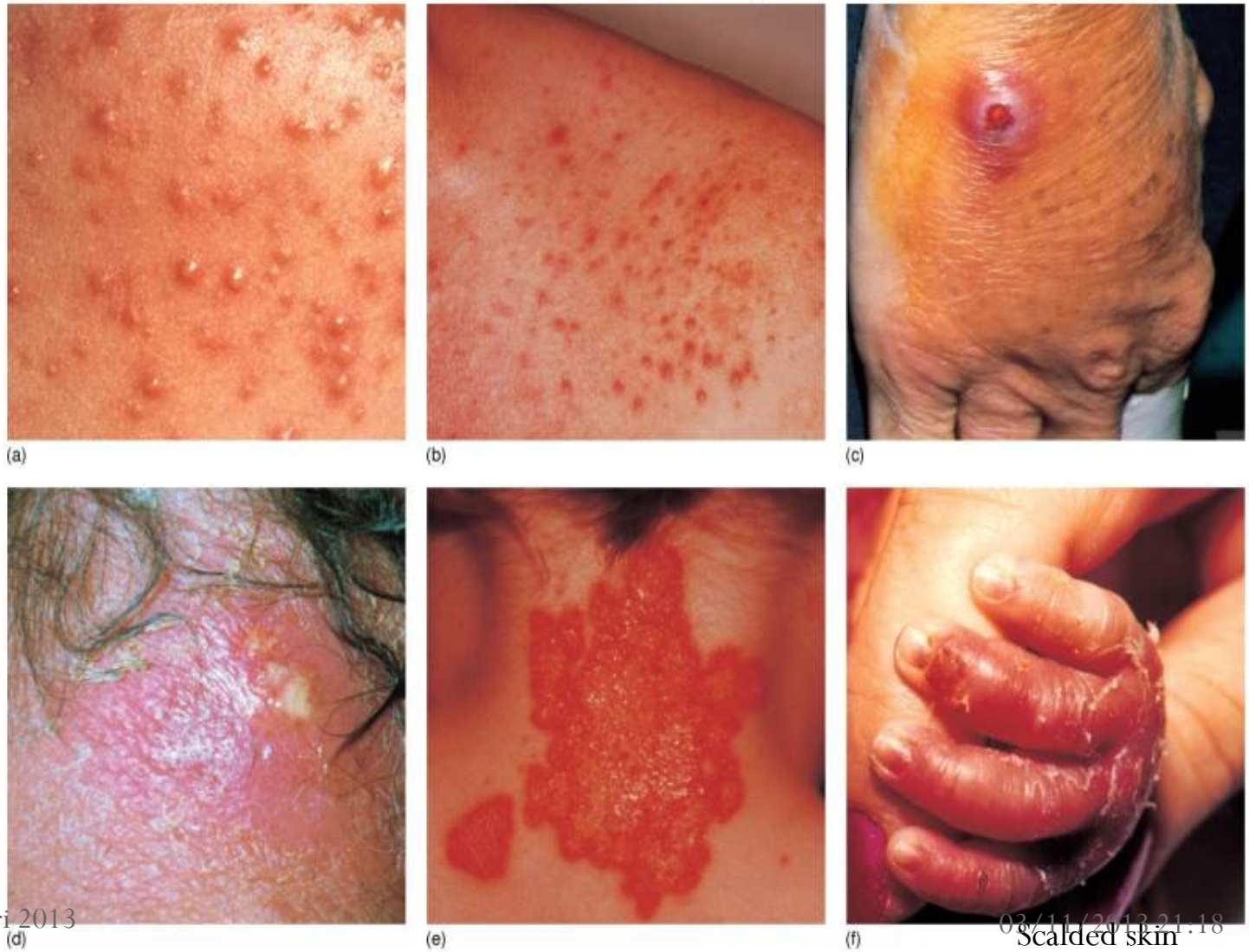


Staph skin infections

Furuncle
(infected hair follicle

Deep folliculitis

superficialfolliculitis



Carbuncle
Multiple abcesses
Around many hair follicles

Staph impetigo

Scalded skin syndrome

Diagnostic Laboratory Tests

- Specimens - pus, blood, tracheal aspirate, or spinal fluid for culture, depending upon the localization of the process.
- Smears - appear as gram positive cocci in clusters in Gram-stained smears of pus or sputum.
- Culture - Specimens planted on blood agar plates give rise to typical colonies in 18 hours at 37 °Cs.

Clinical Concerns and Treatment

- 95% have penicillinase and are resistant to penicillin and ampicillin
- MRSA – methicillin-resistant *S. aureus* – carry multiple resistance
- Some strains have resistance to all major drug groups except vancomycin
- Abscesses have to be surgically perforated
- Systemic infections require intensive lengthy therapy
- Amoxil/cloxacilin, vancomicin

Prevention

- Hand antisepsis is the most important measure in preventing nosocomial infections
- Also important is the proper cleansing of wounds and surgical openings, aseptic use of catheters or indwelling needles, an appropriate use of antiseptics

Staphylococcus epidermidis

- *Staphylococcus epidermidis* is a less common cause of opportunistic infections than *S. aureus*, but is still significant. It is a mediator of nosocomial infections (e.g. catheters, shunts, surgery). It is a major component of the skin flora

Staphylococcus saprophyticus

- This organism is a significant cause of urinary tract infections. It is also coagulase-negative and is not usually differentiated from *S. epidermidis* clinically.

STREPTOCOCCI

General Characteristics of Streptococci

- Gram-positive, spherical arranged in long chains; commonly in pairs
- Non-spore-forming, nonmotile
- Can form capsules and slime layers
- Facultative anaerobes
- Most parasitic forms are **fastidious** and require enriched media
- Small, non-pigmented colonies on culture
- Sensitive to drying, heat, and disinfectants

- Are subdivided into groups by antibodies that recognize surface antigens.
- The most important groupable streptococci are A, B and D.
- Three types of hemolysis reaction (alpha, beta, gamma) are seen after growth of streptococci on sheep blood agar.
- **Alpha** refers to partial hemolysis with a green coloration, **Beta** refers to complete clearing and **gamma** means there is no lysis.
- Group A and group B streptococci are beta hemolytic, whilst D are usually alpha or gamma.
- *Streptococcus pneumoniae* and viridans ("green") streptococci are alpha hemolytic.
- Thus, the hemolysis reaction is important in grouping streptococci.

Human Streptococcal Pathogens

- *S. pyogenes*
- *S. agalactiae*
- Viridans streptococci
- *S. pneumoniae*
- *Enterococcus faecalis*

β-Hemolytic *S. pyogenes* -

- Pyogenes means pus producing
- Individual cocci are spherical or ovoid and are arranged in chains.
- Are non-motile
- Most group A strains produce capsules - impede phagocytosis.
- Are Lancefield Serological Group A
- Have hair-like pili projecting through the capsule - important in the attachment of streptococci to epithelial cells
- **It is the most serious streptococcal pathogen**
- Strict parasite
- Inhabits throat, nasopharynx, occasionally skin

Virulence Factors

Extracellular toxins:

- **Streptolysins** – hemolysins; streptolysin O (SLO) and streptolysin S (SLS) – both cause cell and tissue injury
- **Erythrogenic toxin (pyrogenic)** – induces fever and typical red rash
- **Superantigens** – strong monocyte and lymphocyte stimulants; cause the release of tissue necrotic factor

Extracellular enzymes:

- Streptokinase (fibrinolysin) – digests fibrin clots
- Hyaluronidase – breaks down connective tissue
- DNase – hydrolyzes DNA

Epidemiology

- Humans are the only reservoir
- Inapparent carriers
- Transmission – contact, droplets, food, fomites
- Portal of entry - skin or pharynx
- Children predominant group affected for cutaneous and throat infections
- Systemic infections and progressive sequelae possible if untreated

PATHOGENICITY

1. Skin infections

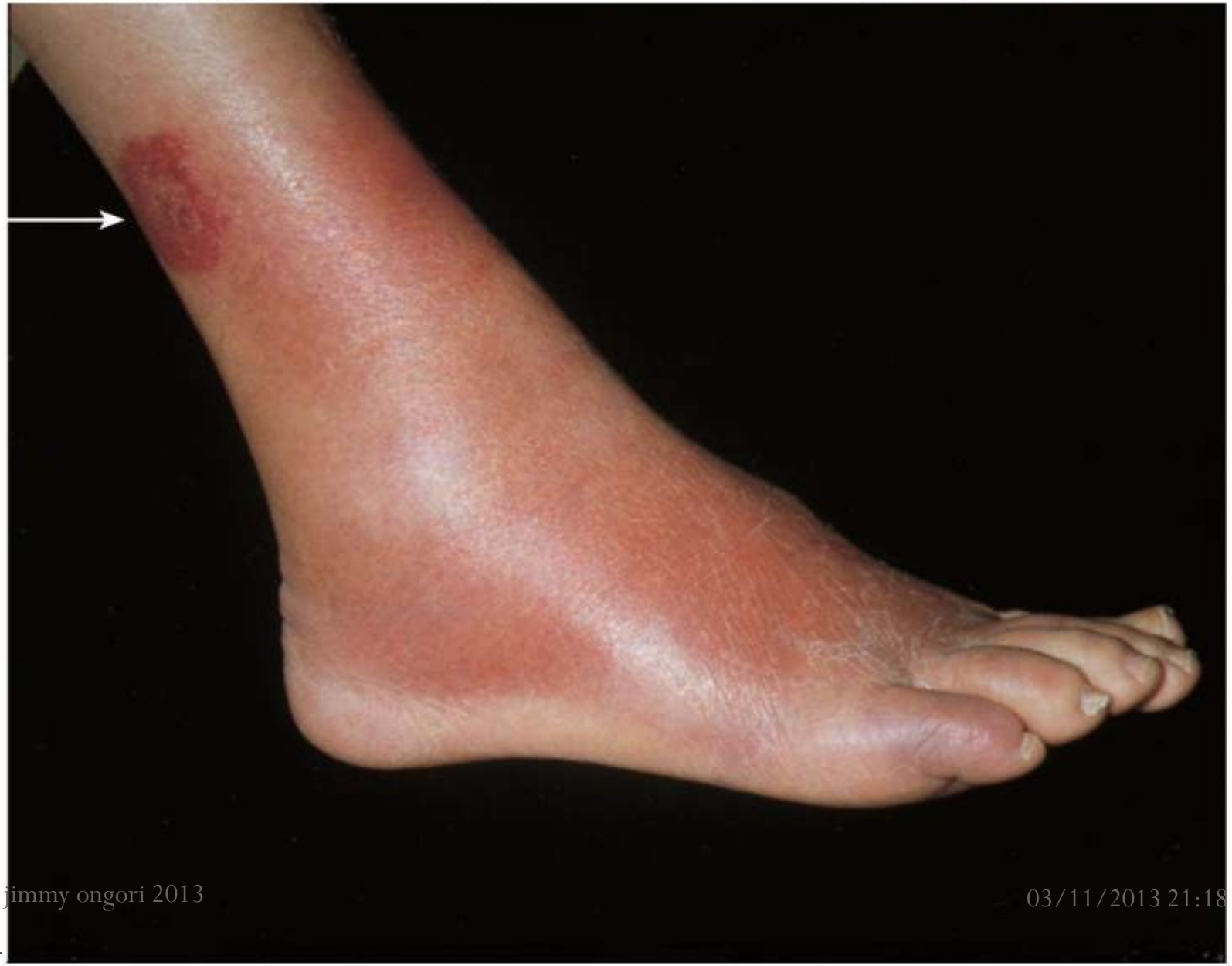
- **Impetigo (pyoderma)** – superficial lesions that break and form highly contagious crust; often occurs in epidemics in school children
- **Erysipelas** – pathogen enters through a break in the skin and eventually spreads to the dermis and subcutaneous tissues; can remain superficial or become systemic

2. Throat infections

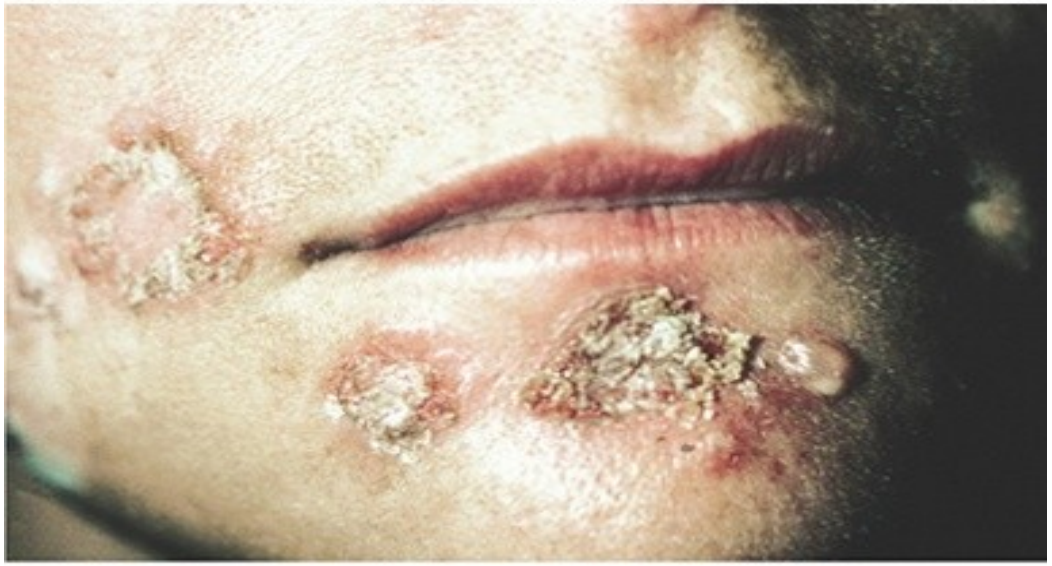
Streptococcal pharyngitis

- The most common infection due to - hemolytic *S pyogenes* is streptococcal sore throat or pharyngitis. *S pyogenes* adhere to the pharyngeal epithelium by means of surface pili.
- The illness may persist for weeks.
- May be characterized by intense nasopharyngitis, tonsillitis, and intense redness and edema of the mucous membranes, with purulent exudate

ERYSIPELAS



Streptococcal skin infections



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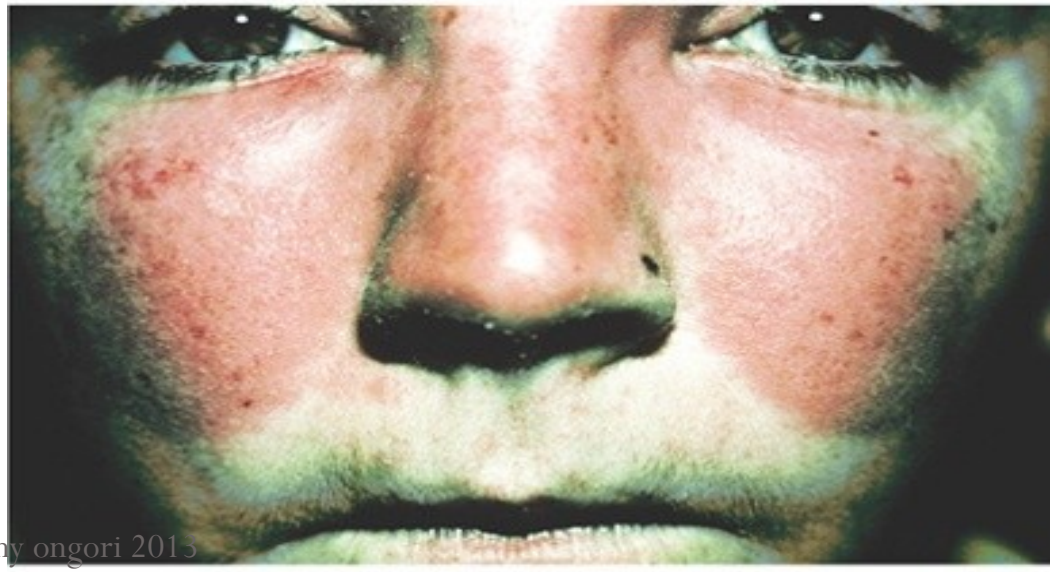

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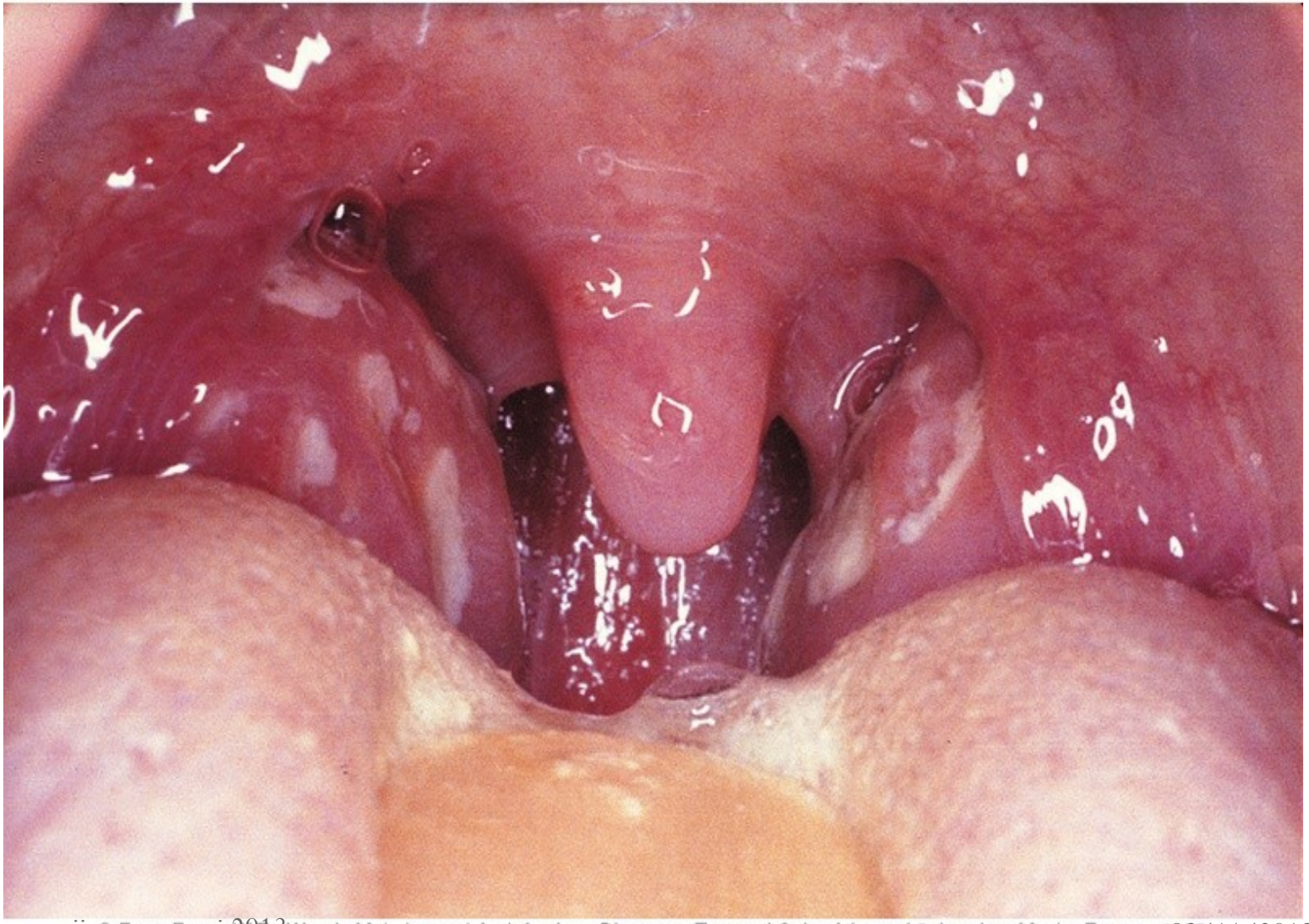
Pharyngitis and tonsillitis



© From Farrar W.E., Woods M.J., Innes J.A.: Infectious Diseases; Text and Color Atlas, ed 2. London, Mosby Europe, 1992.

3. Systemic infections

- **Scarlet fever** – strain of *S. pyogenes* carrying a prophage that codes for pyrogenic toxin; can lead to sequelae
- Septicemia
- Pneumonia
- Streptococcal toxic shock syndrome

Long-Term Complications of Group A Infections

Rheumatic fever - This is the most serious complication because it results in damage to heart muscle and valves.

- Certain strains of group A streptococci contain cell membrane antigens that cross-react with human heart tissue antigens.
- Rheumatic fever is often preceded by *S pyogenes* infection 1–4 weeks earlier

Acute glomerulonephritis – This sometimes develops 3 weeks after *S pyogenes* skin infection

- AGN may be initiated by antigen-antibody complexes on the glomerular basement membrane.

Diagnostic Laboratory Tests

- **Specimens** - depend upon the nature of the infection. A throat swab, pus, or blood for culture. Serum for antibody tests.
- **Smears** - often show single cocci or pairs rather than definite chains.
- **Culture** - on blood agar plates.
- **Antigen Detection Tests -** for rapid detection of group A streptococcal antigen from throat swabs.
- **Serologic Tests -** A rise in the titer of antibodies to many group A streptococcal antigens can be estimated. Such antibodies include antistreptolysin O (ASO) – it is the most widely used.

Treatment

- Penicillin G, erythromycin.
- Antimicrobial drugs are also very useful in preventing reinfection with -hemolytic group A streptococci in rheumatic fever patients.

Prevention

(1) Detection and early antimicrobial therapy of respiratory and skin infections with group A streptococci.

(2) Antistreptococcal chemoprophylaxis in persons who have suffered an attack of rheumatic fever.

α-Hemolytic Streptococci: Viridans Group

- Large complex group
  - *Streptococcus mutans, S. oralis, S. salivarus, S. sanguis, S. milleri, S. mitis*
- Most numerous and widespread residents of the gums and teeth, oral cavity and also found in nasopharynx, genital tract, skin
- Are not very invasive; dental or surgical procedures facilitate entrance leading to; Bacteremia, meningitis, abdominal infection, tooth abscesses

- The most serious infection they cause is – **subacute endocarditis** – blood-borne bacteria settle and grow on heart lining or valves
- Persons with preexisting heart disease are at high risk.
- Colonization of heart by forming biofilms
- *S. mutans* produce slime layers that adhere to teeth - basis for plaque. Involved in dental caries
- Persons with preexisting heart conditions should receive prophylactic antibiotics before surgery or dental procedures.

STREPTOCOCCUS PNEUMONIA

- The pneumococci (*S pneumoniae*) are gram-positive diplococci, lancet-shaped or arranged in chains.
- Causes 60-70% of all bacterial pneumonias
- Posses a capsule of polysaccharide
- Pneumococci are readily lysed by surface-active agents
- Pneumococci are normal inhabitants of the upper respiratory tract of 5–40% of humans and can cause pneumonia, sinusitis, otitis, bronchitis, bacteremia, meningitis, and other infectious processes

Epidemiology and Pathology

- 5-50% of all people carry it as normal flora in the nasopharynx; infections are usually endogenous.
- Very delicate, does not survive long outside its habitat
- Young children, elderly, immune compromised, those with other lung diseases or viral infections, persons living in close quarters are predisposed to pneumonia
- Pneumonia occurs when cells are aspirated into the lungs of susceptible individuals.
- Gains access to middle ear by way of Eustachian tube

PATHOGENICITY

- Cause lobar pneumonia, bronchitis,meningitis, bacteremia, otitis media, sinusitis, conjuctivitis
- Severe infection in the elderly, immune compromised, splenectomy patients
- Pneumonia in children especially in sickle cell disease
- Are normal flora in the upper respiratory tract
- Pneumococci produce disease through their ability to multiply in the tissues.
- They produce no toxins of significance.
- The virulence of the organism is due to capsule, which prevents or delays ingestion by phagocytes.

Diagnostic Laboratory Tests

- Blood, CSF and sputum - smear and culture.
- **Sputum;**
  - Stained Smears - A Gram-stained film shows typical organisms, many polymorphonuclear neutrophils, and many red cells.
  - Culture - The culture is created by sputum cultured on blood agar and incubated in CO_2 or a candle jar. A blood culture is also taken.

Treatment

- Since pneumococci are sensitive to many antimicrobial drugs, early treatment usually results in rapid recovery
- Penicillin G is effective in treating pneumonia caused by pneumococci but would not be effective in treatment of meningitis due to the same strains.
- Some penicillin-resistant strains are resistant to cefotaxime. Resistance to tetracycline and erythromycin occurs also.
- Pneumococci remain susceptible to vancomycin.

Prevention, & Control

- Pneumococcal pneumonia accounts for about 60% of all bacterial pneumonias.
- Predisposing factors are more important than exposure to the infectious agent, and the healthy carrier is more important in spreading pneumococci than the sick patient.
- Vaccines can provide upto 90% protection
- Vaccine is appropriate for elderly, debilitated, or immunosuppressed individuals.
- A pneumococcal conjugate vaccine is currently being given to children <1yr in Kenya

Group B: *Streptococcus agalactiae*

- Regularly resides in human vagina, pharynx and large intestine
- Can be transferred to infant during delivery and cause severe infection
  - most prevalent cause of neonatal pneumonia, sepsis, and meningitis
  - Pregnant women should be screened and treated.
- Wound and skin infections and endocarditis in debilitated people

NEISSERIA

Family Neisseriaceae

- Gram-negative cocci
- Residents of mucous membranes of warm-blooded animals
- Genera include *Neisseria, Moraxella, Acinetobacter.*
- 2 primary human pathogens:
  - *Neisseria gonorrhoeae*
  - *Neisseria meningitidis*

Genus *Neisseria*

- Gram-negative, bean-shaped, diplococci
- None develop flagella or spores.
- Capsules on pathogens
- Pili
- Strict parasites, do not survive long outside of the host
- Aerobic or microaerophilic
- Produce catalase and cytochrome oxidase
- Pathogenic species require enriched complex media and CO_2.

Neisseria gonorrhoeae: The Gonococcus

- The typical Neisseria is a gram-negative, nonmotile diplococcus
- Individual cocci are kidney-shaped; when the organisms occur in pairs, the flat or concave sides are adjacent.
- Found only in man
- Is the causative agent of gonorrhea, the second most common venereal disease.
- **Virulence factors:** pili, other surface molecules for attachment - slows phagocytosis
  - IgA protease – cleaves secretory IgG

- These bacteria grow best on chocolate agar (so-called because it contains heated blood, brown in color); a modified (selective) chocolate agar commonly used is Thayer Martin.

- The colonies are oxidase positive (i.e. produce cytochrome oxidase) which is demonstrated by flooding the plate with a dye which on oxidation changes color.

Epidemiology of Gonorrhea

- Seriously underreported STD
- Found only in humans with strikingly different epidemiological presentations for females and males
- Asymptomatic carriage is a major reservoir
- Transmission primarily by sexual contact
- Lack of protective immunity and therefore reinfection, partly due to antigenic diversity of strains
- Does not survive more than 1-2 hours on fomites

Pathogenesis of Neisseria gonorrhoeae

- Fimbriated cells attach to intact mucus membrane epithelium
- Capacity to invade intact mucus membranes or skin with abrasions
  - Adherence to mucosal epithelium
  - Penetration into and multiplication before passing through mucosal epithelial cells
  - Establish infection in the sub-epithelial layer
- Most common sites of inoculation:
  - Cervix (cervicitis) or vagina in the female
  - Urethra (urethritis) or penis in the male

- Attack mucous membranes of the GUT, eye, rectum, and throat, producing acute suppuration that may lead to tissue invasion; this is followed by chronic inflammation and fibrosis.
- In males - urethritis, with yellow, creamy pus and painful urination. May extend to the epididymis.
- As suppuration subsides in untreated infection, fibrosis occurs, sometimes leading to urethral strictures
- Urethral infection in men can be asymptomatic.

- In females, the 1^0 infection is in the endocervix and extends to the urethra and vagina, giving rise to mucopurulent discharge. May progress to the uterine tubes, causing salpingitis, fibrosis, and obliteration of the tubes.
- Infertility occurs in 20% of women with gonococcal salpingitis.
- Chronic gonococcal cervicitis or proctitis is often asymptomatic.
- Gonococcal bacteremia leads to skin lesions on the hands, forearms, feet, and legs and to tenosynovitis and suppurative arthritis, usually of the knees, ankles, and wrists.

- Gonococcal endocarditis is an uncommon but severe infection.
- Gonococci sometimes cause meningitis and eye infections in adults
- Gonococcal **ophthalmia neonatorum**, an infection of the eye of the newborn, is acquired during passage through an infected birth canal.
- To prevent gonococcal ophthalmia neonatorum, instillation of tetracycline, erythromycin, or silver nitrate into the conjunctival sac of the newborn is compulsory in the United States.

GONORRHEA IN MEN:

Urethritis; Epididymitis

- Most infections among men are acute and symptomatic with purulent discharge & dysuria after 2-5 day incubation period
- Male host seeks treatment early preventing serious sequelae
- The two bacterial agents primarily responsible for urethritis among men are *N. gonorrhoeae* and *Chlamydia trachomatis*

GONORRHEA IN WOMEN

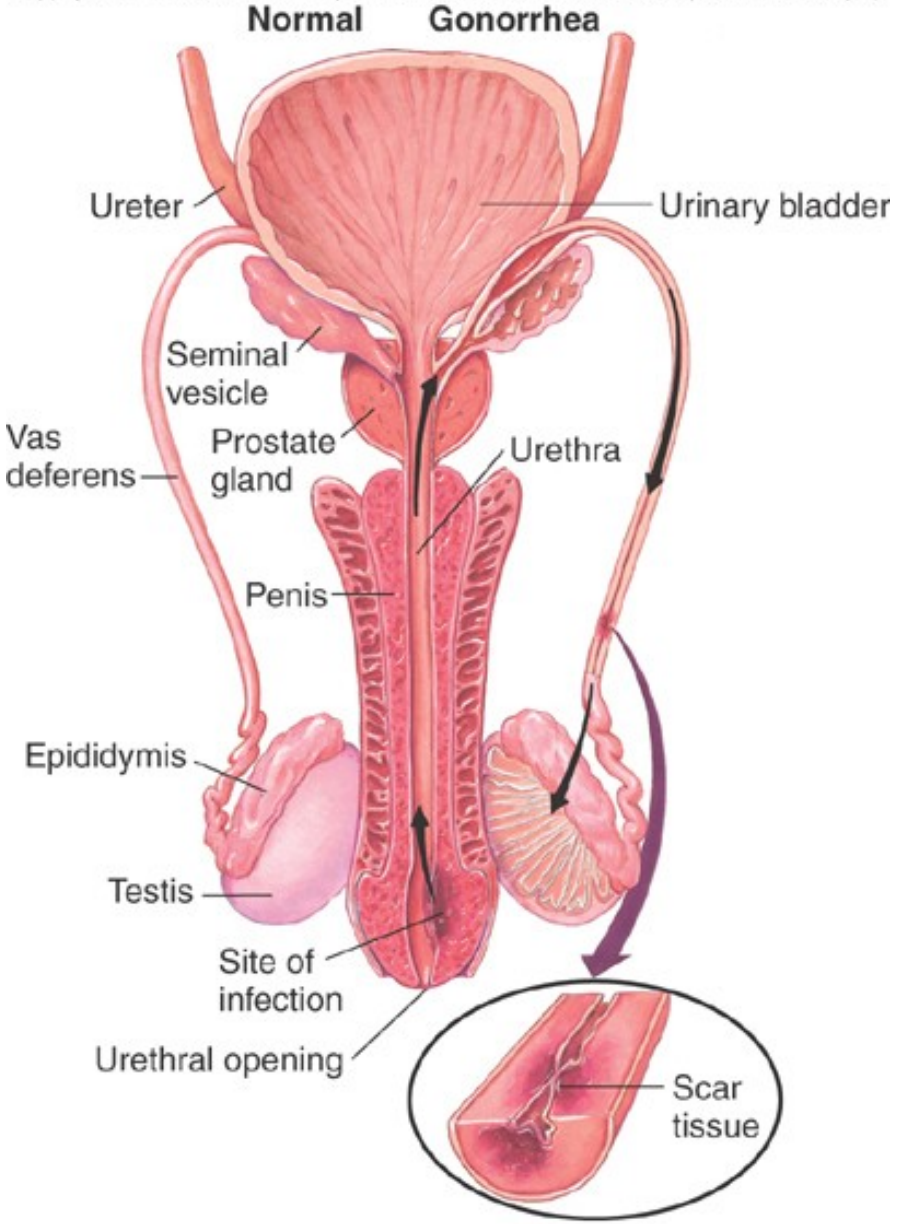
- Cervicitis; Vaginitis; Pelvic Inflammatory Disease (PID); Disseminated Gonococcal Infection (DGI)
- Women are often asymptomatic or have atypical indications (unrecognized S/S)
- Often untreated until PID complications develop
- **Pelvic Inflammatory Disease (PID)**
  - May also be asymptomatic, but difficult diagnosis accounts for many false negatives
  - Can cause scarring of fallopian tubes leading to infertility or ectopic pregnancy

IN WOMEN (cont.) :

Disseminated Gonococcal Infection (DGI):

- Result of gonococcal bacteremia
- Skin lesions
- Petechiae (small, purplish, hemorrhagic spots)
- Pustules on extremities
- Arthralgias (pain in joints)
- Tenosynovitis (inflammation of tendon sheath)
- Septic arthritis
- Occasional complications: Hepatitis; Rarely endocarditis or meningitis

Females	Males
50% risk of infection after single exposure	20% risk of infection after single exposure
Asymptomatic infections frequently not diagnosed	Most initially symptomatic (95% acute)
Major reservoir is asymptomatic carriage in females	Major reservoir is asymptomatic carriage in females
Genital infection primary site is cervix (cervicitis), but vagina, urethra, rectum can be colonized	Genital infection generally restricted to urethra (urethritis) with purulent discharge and dysuria
Ascending infections in 10-20% including salpingitis, tubo-ovarian abscesses, pelvic inflammatory disease (PID) , chronic infections can lead to sterility	Rare complications may include epididymitis, prostatitis, and periurethral abscesses
Disseminated infections more common, including septicemia, infection of skin and joints (1-3%)	Disseminated infections are very rare
Can infect infant at delivery (conjunctivitis, opthalmia neonatorum)	More common in homosexual/bisexual men than in heterosexual populatiuon



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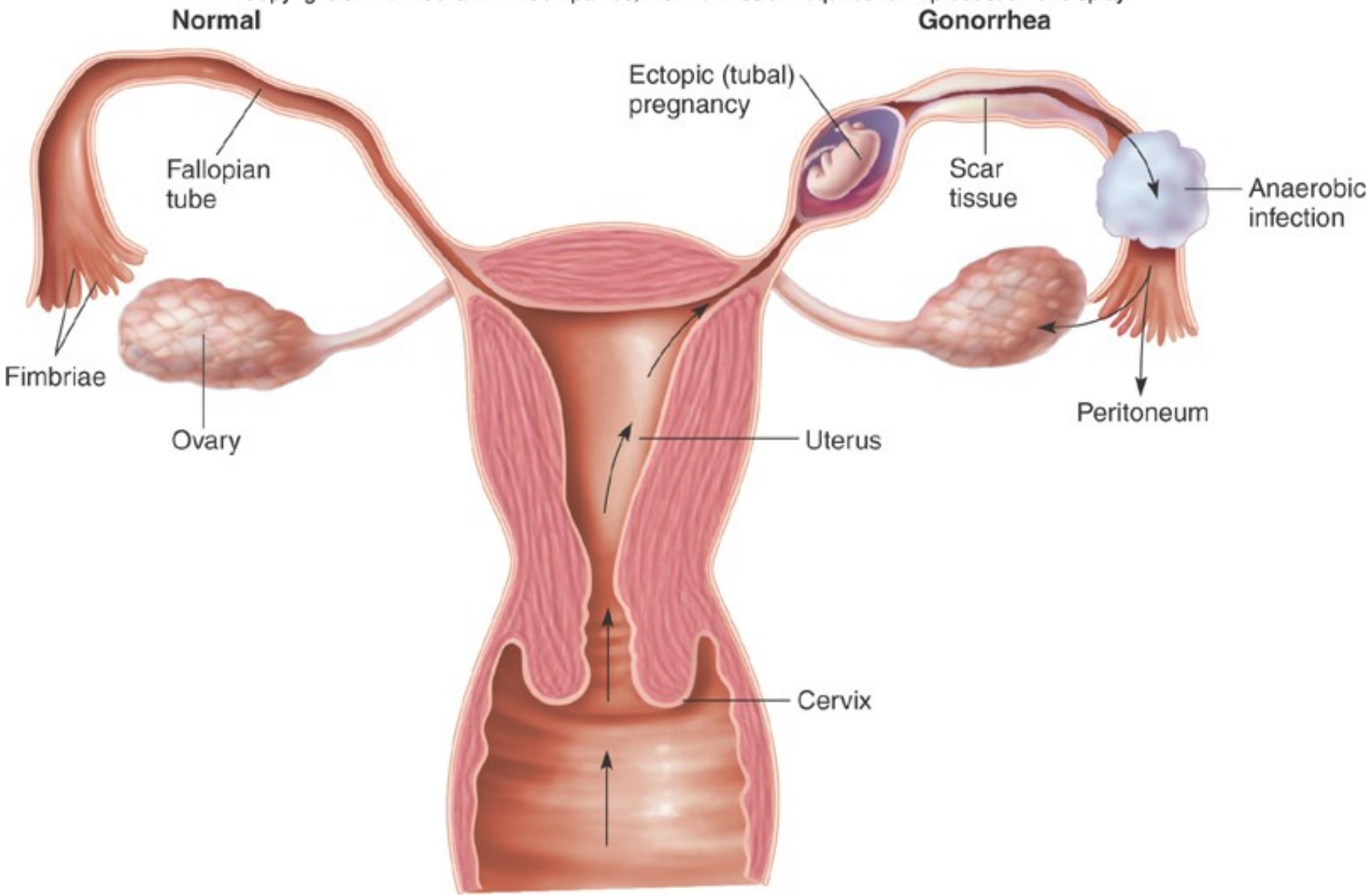


Diagnostic Laboratory Tests

- **Specimens** - Pus and secretions are taken from the urethra, cervix, rectum, conjunctiva, throat, or synovial fluid for culture and smear
- **Smears** - Gram-stained smears - diplococci within pus cells.
- **Culture** on enriched selective medium eg, modified Thayer-Martin medium at 37 °C.
- Oxidase test is positive
- **Nucleic Acid Amplification Tests -** nucleic acid amplification assays are available for direct detection of *N gonorrhoeae* in genitourinary specimens.
- **Serology** - by immunoblotting, radioimmunoassay, and ELISA (enzyme-linked immunosorbent assay) tests

Prevention & Treatment

- Penicillin no longer drug of choice due to resistance
- Uncomplicated infection: ceftriaxone, cefixime or Fluoroquinolone. Can be combined with doxycycline or azithromycin for dual infections with *Chlamydia*
- Chemoprophylaxis of newborns against opthalmia neonatorum with 1% silver nitrate, 1% tetracycline, or 0.5% erythromycin eye ointments
- Treatment of newborns with opthalmia neonatorum with ceftriaxone
- Measures to limit epidemic include education, aggressive detection, and follow-up screening of sexual partners, use of condoms

Neisseria meningitides

- Aerobic, non-spore-forming, Gram-negative diplococci
- Encapsulated (capsule the major virulence factor)
- Cytochrome oxidase positive
- Natural habitat - nasopharyngeal tract of humans .
- 5-15% of the human population carries the bacteria in its nonpathogenic form .
- Also known as meningococcus
- Only infects humans, there is no animal reservoir.

- Oxidase +ve
- Acid production from glucose and maltose, but not sucrose or lactose
- *Neisseria gonorrhoeae*, *N. meningitidis*, and *Moraxella catarrhalis* are **capnophilic** (optimal growth with 3-7% CO_2)
- Growth of *Neisseria meningitidis* occurs on both sheep blood and chocolate agar

Pathogenicity

- Pili-mediated, receptor-specific colonization of nonciliated cells of nasopharynx
- Antiphagocytic polysaccharide capsule allows systemic spread in absence of specific immunity
- Toxic effects mediated by hyperproduction of lipooligosaccharide
- Second most common cause (behind *S. pneumoniae)* of **community-acquired meningitis** in previously healthy adults; swift progression from good health to life-threatening disease

Diseases Associated with *N. meningitidis*

Following dissemination of virulent organisms from the nasopharynx:

- Meningitis
- Septicemia (meningococcemia) with or without meningitis
- Meningoencephalitis
- Pneumonia
- Arthritis
- Urethritis

Epidemiology of Meningococcal Disease

- **Humans only natural hosts**
- **Person-to-person transmission** by aerosolization of respiratory tract secretions in crowded conditions
- **Close contact** with infectious person
- Highest incidence in **children younger than 5 years** and particularly those **younger than 1 year** of age as passive maternal antibody declines
- Commonly **colonize nasopharynx** of healthy individuals; highest oral and nasopharyngeal carriage rates in school-age children, young adults and lower socioeconomic groups

VIRULENCE FACTORS

- Lipooligosaccharide (LOS) is a component of the outer membrane of *N. meningitidis* which acts as an endotoxin which is responsible for fever, septic shock, and hemorrhage due to the destruction of red blood cells.
- A polysaccharide capsule which prevents phagocytosis and aids in evasion of the host immune response
- Fimbriae which mediate attachment of the bacterium to the epithelial cells of the nasopharynx

Pathogenesis of Meningococcal Disease

- Specific receptors for bacterial fimbriae on **nonciliated** columnar epithelial cells in **nasopharynx** of host
- Organisms are **internalized into phagocytic vacuoles, avoid intracellular killing** in absence of humoral immunity and complement system
- **Replicate intracellularly**
- **Hyperproduction of endotoxin (lipid A of LOS)** mediates most clinical manifestations including diffuse vascular damage (e.g., endothelial damage, vasculitis (inflammation of vessel walls), thrombosis (clotting), disseminated intravascular coagulation (DIC)

Laboratory Diagnosis

- Large numbers of encapsulated, small, gram-negative diplococci and polymorphonuclear leukocytes (PMN's) can be seen microscopically in **cerebrospinal fluid**
- Transparent, non-pigmented nonhemolytic colonies on **chocolate blood agar** with **enhanced growth in moist atmosphere with 5% CO_2**
- Oxidase-positive
- Acid production from glucose and maltose but not from other sugars

Laboratory Diagnosis

- **Specimens** – blood, CSF, Nasopharyngeal swab, Puncture material from petechiae may be taken for smear and culture.
- **Smears** - Gram-stained smears show typical neisseriae within polymorphonuclear leukocytes or extracellularly.
- **Culture** – CSF on "chocolate" agar at 37 °C in of 5% CO_2 (candle jar).
  - A modified Thayer-Martin medium with antibiotics (vancomycin, colistin, amphotericin) favors the growth of neisseriae, inhibits many other bacteria, and is used for nasopharyngeal cultures.
- **Serology** - Antibodies to meningococcal polysaccharides can be measured by latex agglutination or hemagglutination tests

Prevention and Treatment

- Penicillin is drug of choice
- Chloramphenicol or cephalosporins as alternatives
- Chemoprophylaxis of close contacts with rifampicin or sulfadiazine (if susceptible)
- Reduction of personal contacts in a population with a high carrier rate. This is accomplished by avoidance of crowding.
- Polyvalent vaccine in people older than 2 years of age for immunoprophylaxis as an adjunct to chemoprophylaxis

BACILLI

Medically Important Gram-Positive Bacilli

Three general groups:

1. **Endospore-formers**

   Bacillus, Clostridium

2. **Non-endospore-formers**

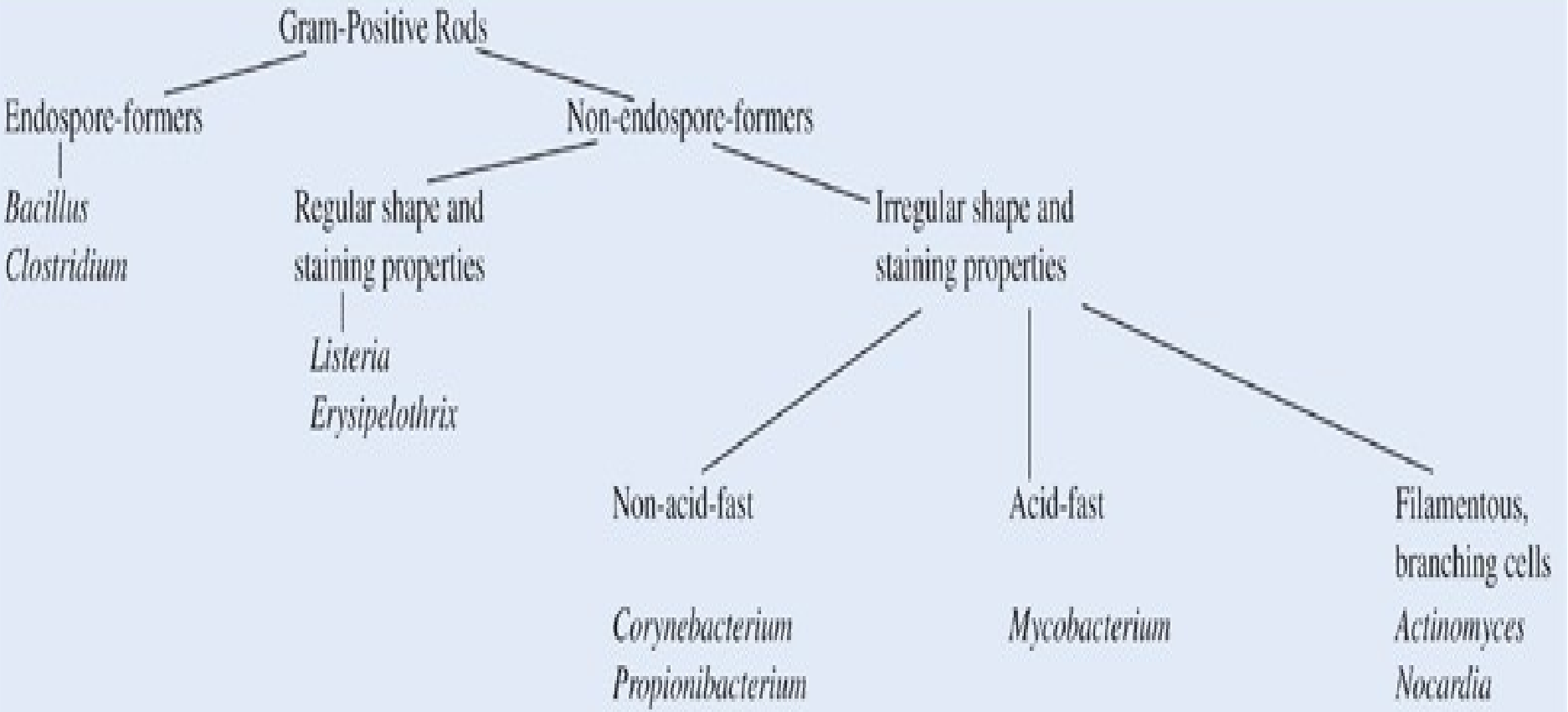
   Listeria, Erysipelothrix

3. **Irregular shaped and staining properties**

   Corynebacterium, Proprionibacterium, Mycobacterium, Actinomyces, Nocardia

TABLE 19.1

Gram-Positive Bacilli



Spore-forming Gram +VE Bacilli

- Genus *Bacillus*
- Genus *Clostridium*

General Characteristics of the Genus *Bacillus*

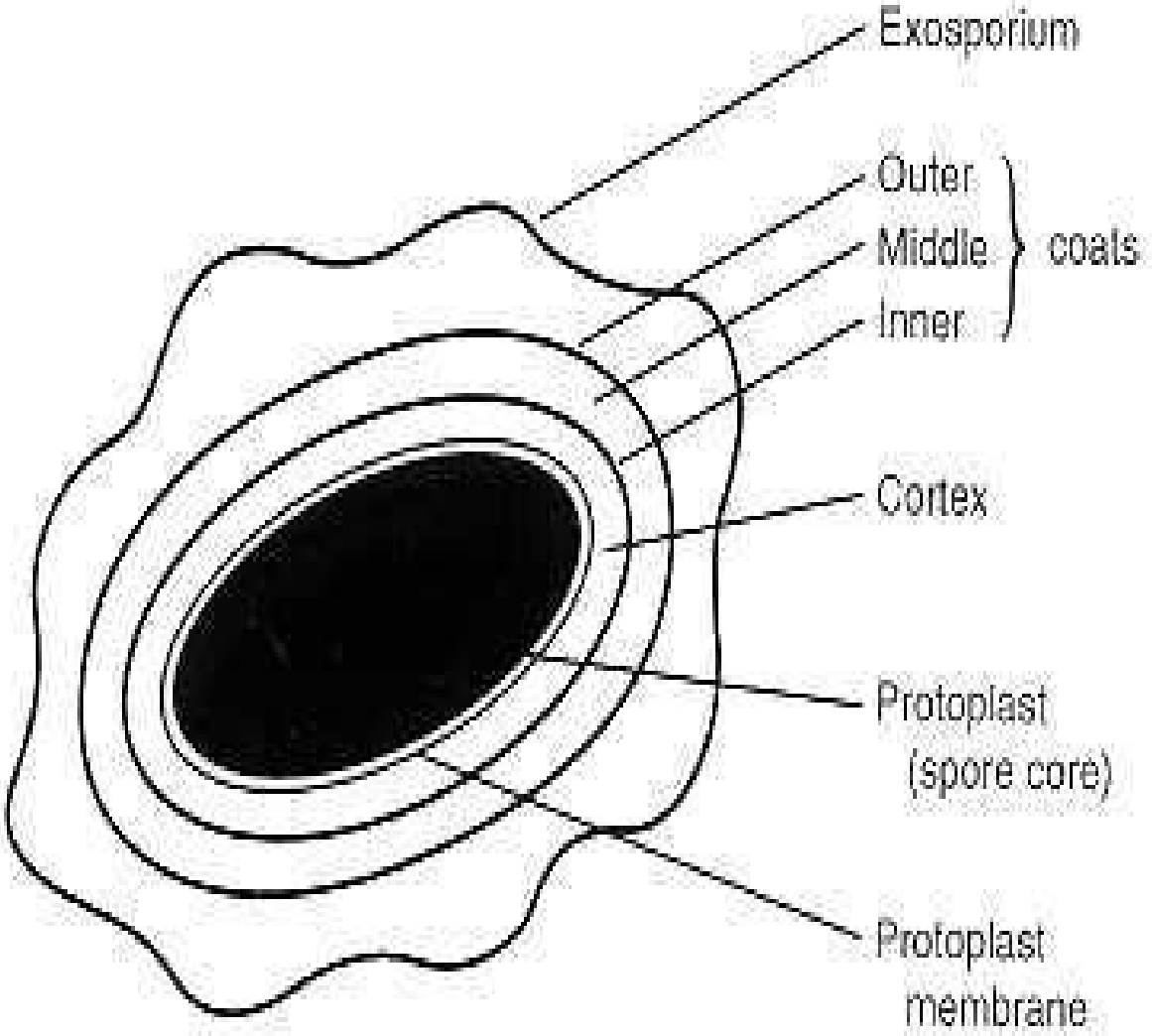
- Gram +VE, endospore-forming, motile rods
- Mostly saprophytic, prevalent in soil, water, and air and on vegetation. *B. cereus* can grow in foods and produce an enterotoxin or an emetic toxin and cause food poisoning
- Aerobic and catalase positive
- Versatile in degrading complex macromolecules
- Primary habitat is soil
- 2 species of medical importance:
  - *Bacillus anthracis*
  - *Bacillus cereus*

Bacillus anthracis

- Large, block-shaped rods
- Gram +VE rod
- Facultative anaerobe
- Central spores that develop under all conditions except in the living body
- Virulence factors – polypeptide capsule and exotoxins

Endospore

- Oxygen required for sporulation
- 1 spore per cell
- Dehydrated cells
  - Highly resistant to heat, cold, chemicals, disinfectants, dry periods
- Protoplast carries the material for future vegetative cell
- Cortex provides heat and radiation resistance
- Spore wall provides protection from chemicals & enzymes


Exosporium
Outer
Middle
Inner
coats
Cortex
Protoplast
(spore core)
Protoplast
membrane

Epidemiology of *Bacillus anthracis*

- Anthrax is primarily a disease of herbivores—goats, sheep, cattle, horses, etc
- Enzootic in certain countries (e.g., Turkey, Iran, Pakistan,and Sudan)
- Anthrax spores are infectious for decades
  - Used Biologic warfare experiments - bioterrorism
- Three well-defined cycles
  - Survival of spores in the soil
  - Animal infection
  - Infection in humans

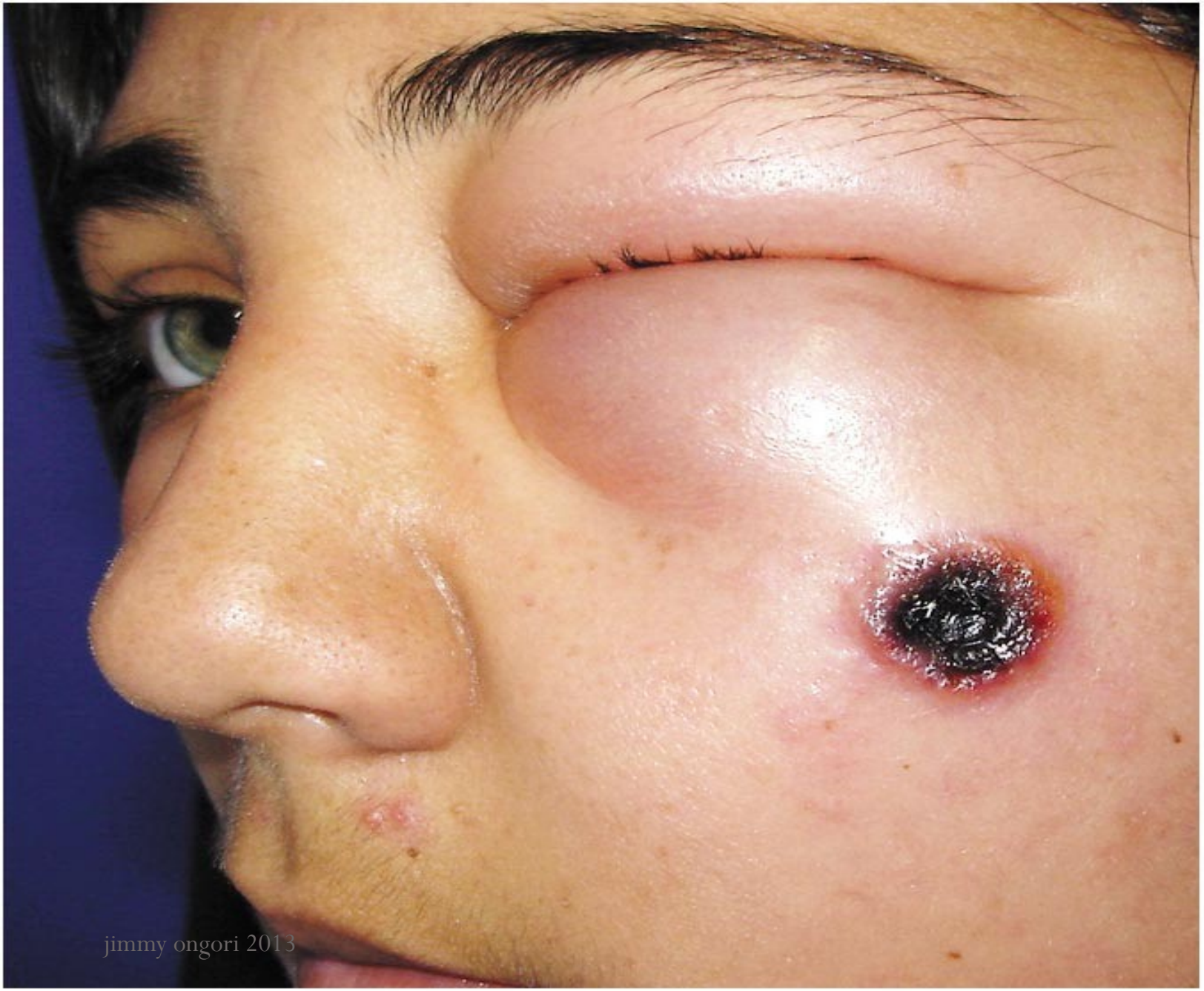
Epidemiology of *Bacillus anthracis (cont.)*

- Primarily a disease of herbivorous animals
- Most commonly transmitted to humans by direct contact with animal products (e.g., wool and hair)
- Also acquired via inhalation & ingestion
  - Increased mortality with these portals of entry
- Still poses a threat
  - Importing materials contaminated with spores (e.g., bones, hides, and other materials)
  - Usually encountered as an occupational disease among Veterinarians, agricultural workers

Clinical Presentation of Anthrax

1. Cutaneous Anthrax

- 95% human cases are cutaneous infections
- 1 to 5 days after contact
- Small, pruritic, non-painful papule at inoculation site, Papule develops into hemorrhagic vesicle & ruptures
- Slow-healing painless ulcer covered with black eschar surrounded by edema
- Infection may spread to lymphatics
- Septicemia may develop
- 20% mortality in untreated cutaneous anthrax



2. Inhalation Anthrax

- Virtually 100% fatal (pneumonic)
- Meningitis may complicate cutaneous and inhalation forms of disease
- Pharyngeal anthrax
    - Fever
    - Pharyngitis
    - Neck swelling

3. Gastrointestinal (Ingestion) Anthrax

- Virtually 100% fatal
- Abdominal pain
- Hemorrhagic ascites
- Paracentesis fluid may reveal gram-positive rods

Laboratory Diagnosis

- Gram stain
- Culture of *B. anthracis* from the blood, skin lesions, vesicular fluid, or respiratory secretions
- Rapid detection methods

  - PCR for detection of nucleic acid

  - ELISA assay for antigen detection

  - Other immunohistochemical and immunoflourescence examinations

Treatment and Prevention

- Penicillin is drug of choice
- Ciprofloxacin, Erythromycin, chloramphenicol
- Doxycycline now commonly recognized as prophylactic
- Soil is contaminated with anthrax spores from the carcasses of dead animals. These spores remain viable for decades. Control measures include
  - Disposal of animal carcasses - burning or by deep burial in lime pits
  - Decontamination of animal products
  - Protective clothing and gloves for handling potentially infected materials
  - Immunization of domestic animals and Persons with high occupational risk.

Bacillus cereus

- Gram +ve bacilli
- Spore forming
- Facultative anaerobe
- Non-fastidious growth requirements

Virulence

- Enterotoxin
- Spores can survive in soil
- Tissue destruction mediated by cytotoxic enzymes – cereolysin and phospholipase C

Epidemiology

- Ubiquitous in soils throughout the world
- People at risk include those who consume food contaminated with the bacterium (eg rice, meat, vegetables, sauces), those with penetrating injuries and those who receive intravenous injections

Diseases

- Infections include emetic (vomiting) and diarrheal forms of GE, ocular infection after trauma to the eye and other opportunistic infections

Diagnosis

- Isolation of the organism in implicated food product or nonfecal specimen (eg eye, wound)

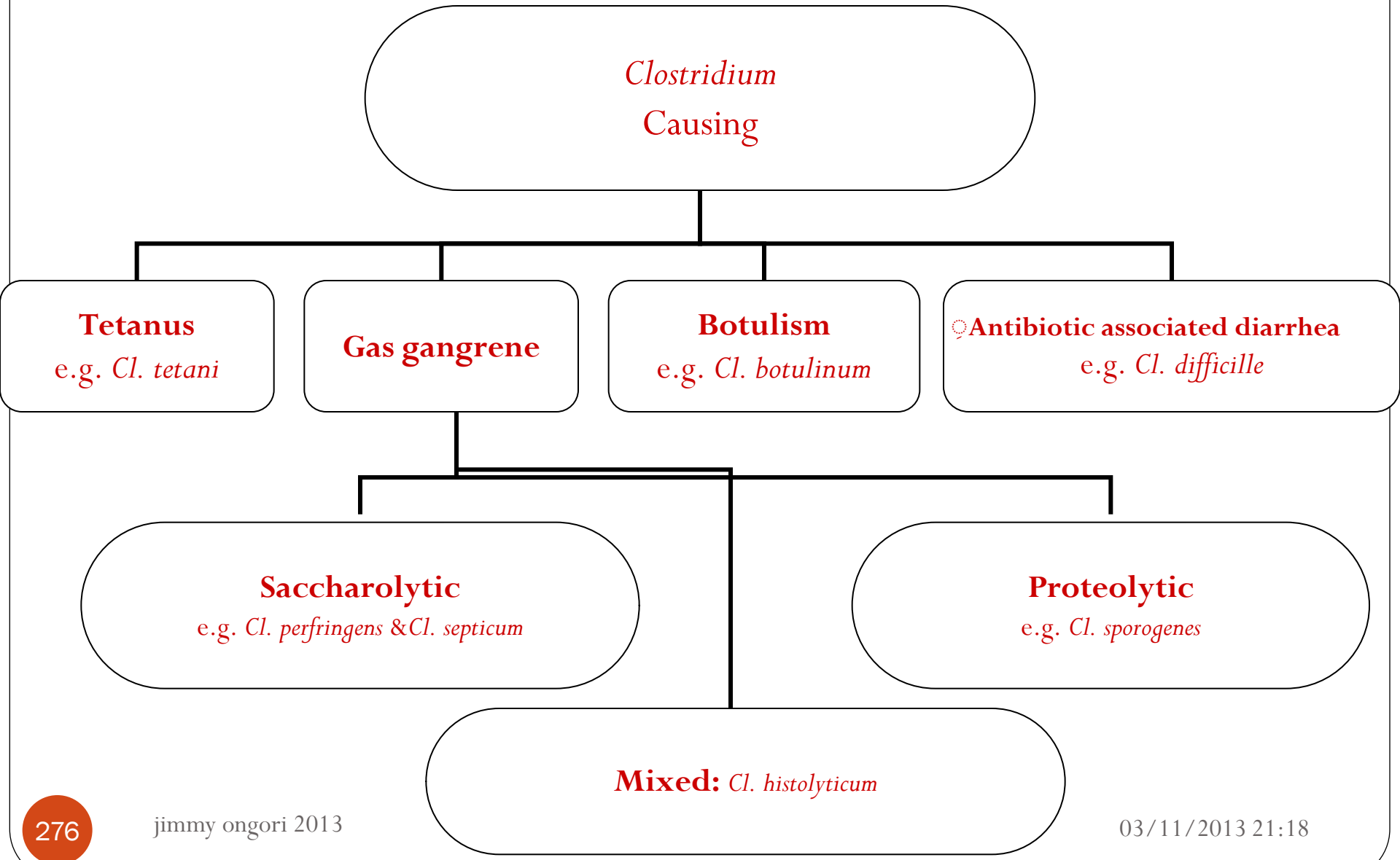
Treatment, Prevention, and Control

- GIT infections treated symptomatically
- Ocular or other invasive diseases – vancomycin, clindamycin, ciprofloxacin, or gentamicin
- GIT disease prevented by proper preparation of food

CLOSTRIDIA

- Large Gram +VE, Straight or slightly curved rods with slightly rounded ends
- Anaerobic
- Spore bearing
- Saprophytes
- Some are commensals of the animal & human gut which invade the blood and tissue when host die and initiate the decomposition of the corpse
- Causes diseases such as gas gangrene, tetanus, botulism & pseudo-membranous colitis by producing toxins which attack the neurons pathways

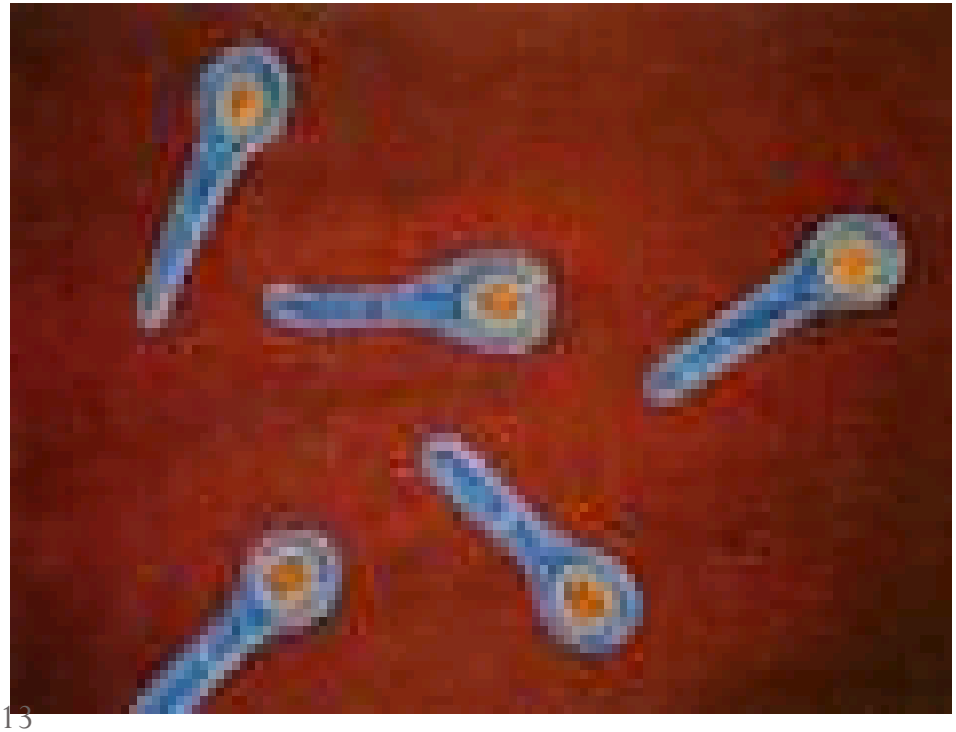
Clostridia of medical importance



CLOSTRIDIUM CAUSING TETANUS
Clostridium tetani

- Gram +ve, straight, slender rod with rounded ends
- All species form endospores (drumstick with a large round end)
- Fermentative
- Obligate anaerobe
- Motile by peritrichous flagella
- Grows well in cooked meat broth and produces a thin spreading film when grown on enriched blood agar

- Spores are highly resistant to adverse conditions
- Spores do not germinate and growth does not normally proceed unless a suitably low redox potential exists
- Iodine (1%) in water is able to kill the spores within a few hours



Toxins

Cl. tetani produces two types of toxins:

- **Tetanolysin**, which causes lysis of RBCs
- **Tetanospasmin** is a neurotoxin and essential pathogenic product
- Tetanospasmin is toxic to humans and various animals when injected parenterally, but it is not toxic by the oral route
- Tetanospasmin causes increasing excitability of spinal cord neurons and muscle spasm

Pathogenesis

- *C tetani* is not an invasive organism - Infection remains strictly localized in the area of devitalized tissue (wound, burn, injury, umbilical stump, surgical suture) into which the spores have been introduced.
- Germination of the spore and development of vegetative organisms that produce toxin are aided by (1) necrotic tissue, (2) calcium salts, and (3) associated pyogenic infections, all of which aid establishment of low oxidation-reduction potential.
- The toxin released from vegetative cells reaches the central nervous system and rapidly becomes fixed to receptors in the spinal cord and brain stem.

Clinical Findings

- Incubation period 4–5 days to weeks.
- Characterized by tonic contraction of voluntary muscles. Muscular spasms often involve first the area of injury and infection and then the muscles of the jaw (trismus, lockjaw), which contract so that the mouth cannot be opened.
- Gradually, other voluntary muscles become involved, resulting in tonic spasms. Any external stimulus may precipitate a tetanic generalized muscle spasm.
- The patient is fully conscious, and pain may be intense. Death usually results from interference with the mechanics of respiration.
- The mortality rate in generalized tetanus is very high.

Laboratory Diagnosis of Tetanus

- The diagnosis of tetanus depends primarily upon the clinical manifestation of tetanus including muscle spasm and rigidity.
- Specimen: Wound exudates using capillary tube
- Culture:
  - On blood agar and incubated anaerobically
  - Growth appears as a fine spreading film.
- Gram stain
  - *Cl. tetani* is Gram positive rod motile with a round terminal spore giving a drumstick appearance

Prevention & Treatment

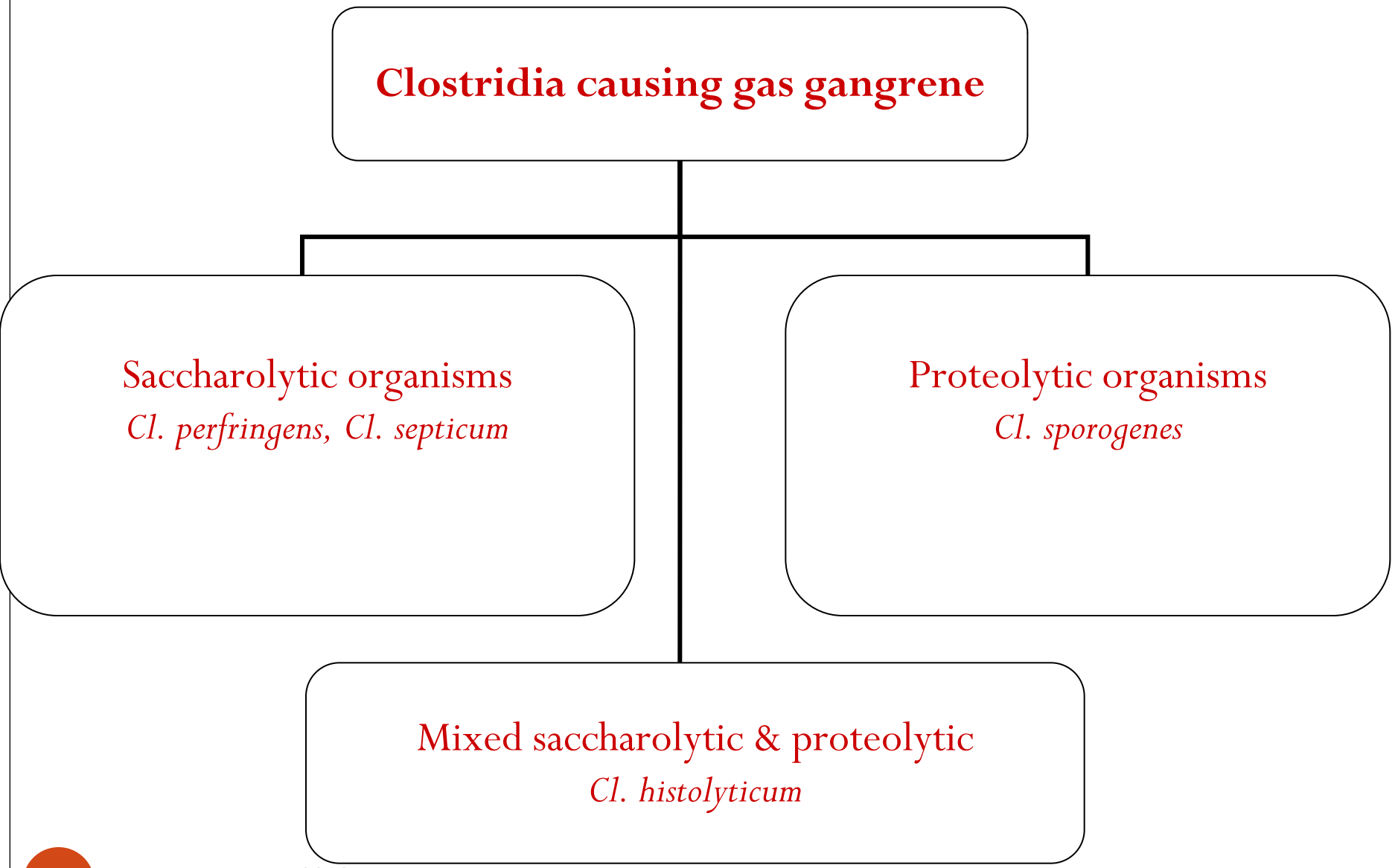
- Muscle relaxants, sedation, and assisted ventilation for patients with symptoms
- Prevention is most important and depends upon;
  - active immunization with toxoids - TT
  - proper care of wounds contaminated with soil, etc
  - prophylactic use of antitoxin
  - administration of penicillin.
- Human antitoxin (tetanus immune globulin) gives adequate systemic protection - It neutralizes the toxin that has not been fixed to nervous tissue.

- Surgical debridement is vitally important because it removes the necrotic tissue that is essential for proliferation of the organisms.
- Penicillin strongly inhibits the growth of *C tetani* and stops further toxin production.
- Antibiotics may also control associated pyogenic infection.
- When a previously immunized individual sustains a potentially dangerous wound, an additional dose of toxoid should be injected to restimulate antitoxin production.

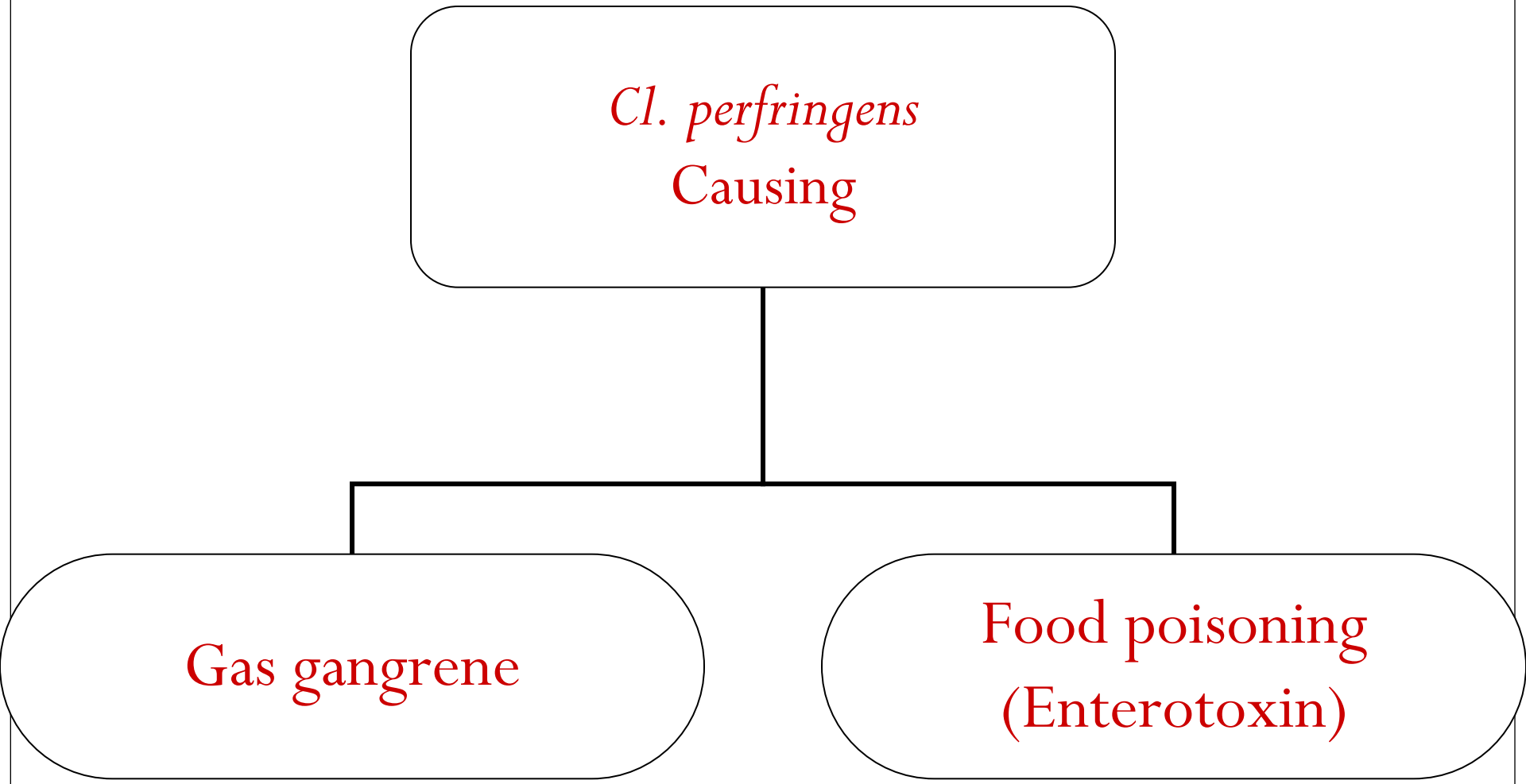
Control

- Tetanus is a totally preventable disease.
- Immunization with tetanus toxoid
- Tetanus toxoid is produced by detoxifying the toxin with formalin and then concentrating it.
- In young children, tetanus toxoid is often combined with diphtheria toxoid, pertussis, Hepatitis B and Haemophilus influenza vaccine (Pentavalent) and given at 6 weeks, 10 weeks and 14 weeks of life, previously given as DPT
- Control measures are not possible because of the wide dissemination of the organism in the soil and the long survival of its spores.

Clostridium Causing Gas Gangrene



Saccharolytic Microorganisms



Clostridium perfringens

- Large Gram-positive bacilli with stubby ends
- Capsulated
- Non motile (*Cl. tetani* is motile)
- Anaerobic
- Grown quickly on selective media
- Can be identified by Nagler reaction
- *Clostridium perfringens* most frequent clostridia involved in soft tissue and wound infections - **myonecrosis**
- Spores found in soil, human skin, intestine, and vagina
- Predisposing factors – surgical incisions, compound fractures, diabetic ulcers, septic abortions, puncture wounds, gunshot wounds

Toxins

- α toxin (phospholipase C, lecithinase) is the most important toxin
  - Lyses of RBCs, platelets, leucocytes and endothelial cells
  - Increased vascular permeability with massive hemolysis and bleeding, tissue destruction
  - Hepatic toxicity and myocardial dysfunction
- β-toxin is responsible for necrotic lesions in necrotizing enterocolitis
- Enterotoxin is heat labile toxin produced in colon → food poisoning

Pathology

- Not highly invasive; requires damaged and dead tissue and anaerobic conditions
- Conditions stimulate spore germination, vegetative growth and release of exotoxins, and other virulence factors.
- Fermentation of muscle carbohydrates results in the formation of gas and further destruction of tissue.

Clinical Diseases

1.Soft tissue infections - Portal of entry: trauma or intestinal tract.

- Usually caused by mixed infection including toxigenic clostridia, proteolytic clostridia and various cocci and gram-negative organisms.Three types of infections:
  - Cellulitis: gas formation in the soft tissue.
  - Fasciitis or suppurative myositis: accumulation of gas in the muscle planes.
  - Myonecrosis or gas gangrene: a life-threatening disease.

2. Gas gangrene

- Spores germinate, vegetative cells multiply, ferment carbohydrates and produce gas in the tissue. This results in distension of tissue and interference with blood supply
- Bacteria produce necrotizing toxin and hyaluronidase, which favor the spread of infection, tissue necrosis extends, resulting in increased bacterial growth, hemolytic anemia, then severe toxemia and death.

Incubation: 1-7 days after infection.

Can be also caused by other *Clostridium* species.

3. Food poisoning

The enterotoxin causes marked hypersecretion in jejunum and ileum.

Enterotoxin: a heat-labile protein produced by some strains of *C. perfringens* type A. When $>10^8$ cells in contaminated meat are ingested and sporulate in the small intestine, enterotoxin is formed.

- It disrupts ion transport in the enterocytes
- Symptoms: diarrhea, usually without vomiting or fever.

Laboratory Diagnosis

- **Specimen:** Histological specimen/wound exudates
- **Microscopical examination (Gram, Spore stain etc)**
  - Gram-positive bacilli, non motile, capsulated & sporulated
  - The spore is oval, sub-terminal & non bulging
  - Spores are rarely observed
- **Culture:** Anaerobically at 37^0C
  - **On Robertson's cooked meat medium →** blackening of meat will observed with the production of H_2S and NH_3
  - **On blood agar →** β-hemolytic colonies

Treatment and Prevention

- Immediate cleansing of dirty wounds, deep wounds, decubitus ulcers, compound fractures, and infected incisions
- Debridement of disease tissue
- Large doses of cephalosporin or penicillin
- Hyperbaric oxygen therapy
- No vaccines available

Clostridium difficile-Associated Disease

- Normal resident of colon, in low numbers
- Causes antibiotic-associated colitis
  - relatively non-invasive; treatment with broad-spectrum antibiotics kills the other bacteria, allowing *C. difficile* to overgrow
- Produces enterotoxins that damage intestines
- Major cause of diarrhea in hospitals
- Increasingly more common in community acquired diarrhea

Treatment and Prevention

- Mild uncomplicated cases respond to fluid and electrolyte replacement and withdrawal of antimicrobials.
- Severe infections treated with oral vancomycin or metronidazole and replacement cultures
- Increased precautions to prevent spread

Clostridial Food Poisoning

- *Clostridium botulinum* – rare but severe intoxication usually from home canned food
- *Clostridium perfringens* – mild intestinal illness; second most common form of food poisoning worldwide

BOTULINUM FOOD POISONING - BOTULISM

- Botulism – intoxication associated with inadequate food preservation

Clostridium botulinum

- Gram positive, Spore-forming, Obligate anaerobic bacillus
  - Spores
    - Ubiquitous, Resistant to heat, light, drying and radiation
- Commonly inhabits soil and water

Neurotoxins

- Seven different types: A - G
  - All cause flaccid paralysis
  - Only a few nanograms can cause illness
  - Binds neuromuscular junctions
- Toxin: Destroyed by boiling
- Spores: Higher temperatures to be inactivated

Pathogenesis

- Spores are present on food when gathered and processed.
- Anaerobic conditions favor spore germination and vegetative growth.
- Potent toxin, **botulin**, is released.
- Toxin is carried to neuromuscular junctions and blocks the release of acetylcholine, necessary for muscle contraction to occur.
- Double or blurred vision, difficulty swallowing, neuromuscular symptoms

Transmission

- Ingestion of – Organism, Spores, Neurotoxin
- Wound contamination
- Inhalation

Human Disease

- Three forms
  - Foodborne
  - Wound
  - Infant
- All forms fatal and a medical emergency
- Incubation period: 12-36 hours

1.Foodborne Botulism

- Preformed toxin ingested from contaminated food
- Most common from home-canned foods
  - Asparagus, green beans, beets, corn, baked potatoes, garlic, chile peppers, tomatoes

2.Infant Botulism

- Spore ingestion - Germinate then toxin released and colonize large intestine
- Infants < 1 year old, but mostly < 6 months old
- Spores from varied sources - Honey, food, dust, corn syrup

3. Wound Botulism

- Organism enters wound
  - Develops under anaerobic conditions
  - From ground-in dirt or gravel
  - It does not penetrate intact skin

Prevention

- Do not feed honey to children <1 yr of age
- Proper food preservation methods
  - Proper time, temperature and pressure
    - 80^{o}C for 30 min or 100^{o}C for 10 min
- Prompt refrigeration of foods
- Boil foods for > 10 minutes
- Decontamination
  - Boil suspected food before discarding
  - Boil or chlorine disinfect utensils used

Clostridial Gastroenteritis

- *Clostrium perfringens*
- Spores contaminate food that has not been cooked thoroughly enough to destroy spores.
- Spores germinate and multiply (especially if unrefrigerated).
- When consumed, toxin is produced in the intestine; acts on epithelial cells, acute abdominal pain, diarrhea, and nausea
- Rapid recovery

Gram-Positive Non-Spore-Forming Bacilli

Medically important genera:

- *Corynebacterium*
- *Proprionibacterium*
- *Mycobacterium*
- *Actinomyces*
- *Nocardia*
- *Listeria monocytogenes*
- *Erysipelothrix rhusiopathiae*

CORYNEBACTERIA (Genus *Corynebacterium*)

- Gram positive bacilli
- Aerobic or facultatively anaerobic
- Small, pleomorphic (club-shaped), gram-positive bacilli that appear in short chains (“V” or “Y” configurations) or in clumps resembling “Chinese letters”
- Cells contain metachromatic granules
- Lipid-rich cell wall contains meso-diaminopimelic acid, arabino-galactan polymers, and short-chain mycolic acids

Pathogenic Corynebacterial Species

- ***Corynebacterium diphtheriae***
- ***Corynebacterium jeikeium***
- ***Corynebacterium urealyticum***

Corynebacterium diphtheriae

- Aerobic, Gram positive, Noncapsulated, rods
- Gray-black colonies on medium
- Metachromatic granules
- Reservoir of healthy carriers; potential for diphtheria is always present
- Most cases occur in non-immunized children living in crowded, unsanitary conditions.
- Acquired via respiratory droplets from carriers or actively infected individuals

Transmission

- Solely among humans
- Spread by droplets
- Secretions
- Direct contact

Risk factors

- Poor nutrition
- Crowded or unsanitary living conditions
- Low vaccine coverage among infants and children
- Immunity gaps in adults

Diphtheria toxin

- Is a heat-labile polypeptide that can be lethal in a dose of 0.1 g/kg.
- It is assumed that the abrupt arrest of protein synthesis is responsible for the necrotizing and neurotoxic effects of diphtheria toxin.
- Toxin diffuses throughout body via blood
  - Cardiac, neurologic complications
  - Heart/respiratory damage, paralysis

Pathology

- Diphtheria toxin is absorbed into the mucous membranes and causes destruction of epithelium and a superficial inflammatory response.
- A grayish "pseudomembrane" is formed—commonly over the tonsils, pharynx, or larynx.
- The regional lymph nodes in the neck enlarge, and there may be marked edema of the entire neck.
- The diphtheria bacilli within the membrane continue to produce toxin actively. This is absorbed and results in distant toxic damage
- Nerve damage, resulting often in paralysis of the soft palate, eye muscles, or extremities.

Diagnostic Laboratory Tests

- Dacron swabs from the nose, throat, or other lesions. Swabs should be collected from beneath any visible membrane.
- Smears stained with alkaline methylene blue or Gram stain show beaded rods in typical arrangement.
- Loeffler slant may yield organisms of typical "diphtheria-like" morphology. In 36–48 hours. Colonies on tellurite medium are sufficiently definite for recognition of *C diphtheriae*.
- Toxigenicity tests
- PCR, ELISA

Control

- **Sanitary:** Reduce carrier rate by use of vaccine.
- **Immunological:** A vaccine (Pentavalent) prepared from an alkaline formaldehyde inactivated toxin (i.e. toxoid) is required.
- Passive immunization with antitoxin can be used for patients.
- **Chemotherapeutic:** Penicillin, erythromycin or gentamicin are drugs of choice.

Mycobacteria: Acid-Fast Bacilli

- *Mycobacterium tuberculosis*
- *M. leprae*
- *M. avium* complex
- *M. fortuitum*
- *M. marinum*
- *M. scrofulaceum*
- *M. paratuberculosis*

Genus *Mycobacterium*

- Gram-positive irregular bacilli
- Acid-fast staining
- Strict aerobes
- Produce catalase
- Possess mycolic acids and a unique type of peptidoglycan
- Do not form capsules, flagella or spores
- Grow slowly

Mycobacterium tuberculosis

- Tubercle bacillus
- Once stained they resist decolorization by acid or alcohol and are therefore called "acid-fast" bacilli.
- Mycobacteria cannot be classified as either gram-positive or gram-negative. Once stained by basic dyes they cannot be decolorized by alcohol, regardless of treatment with iodine.
- Produces no exotoxins or enzymes that contribute to infectiousness
- Virulence factors - contain complex waxes and cord factor that prevent destruction by lysosomes or macrophages
- ***Mycobacterium tuberculosis*** is the etiologic agent of tuberculosis in humans. Humans are the only reservoir for the bacterium.
- Chains of cells in smears made from in vitro-grown colonies often form distinctive serpentine cords

Cell Wall Structure

- The cell wall structure is unique among prokaryotes, and it is a major determinant of virulence for the bacterium.
- The cell wall complex contains **peptidoglycan**, but otherwise it is composed of complex lipids. Over 60% of the mycobacterial cell wall is lipid. The lipid fraction of MTB's cell wall consists of three major components, mycolic acids, cord factor, and wax-D.

i. **Mycolic acids** are unique alpha-branched lipids found in cell walls of *Mycobacterium* and *Corynebacterium*. They make up 50% of the dry weight of the mycobacterial cell envelope. Mycolic acids are strong hydrophobic molecules that form a lipid shell around the organism and affect permeability properties at the cell surface. Mycolic Acids are thought to be a significant determinant of virulence in MTB.

ii. **Cord Factor** is responsible for the serpentine cording mentioned above. Cord factor is toxic to mammalian cells and is also an inhibitor of PMN migration. Cord factor is most abundantly produced in virulent strains of MTB.

iii. **Wax-D** in the cell envelope is the major component of **Freund's complete adjuvant** (CFA).

The high concentration of lipids in the cell wall of *Mycobacterium tuberculosis* have been associated with these properties of the bacterium:

- Impermeability to stains and dyes
- Resistance to many antibiotics
- Resistance to killing by acidic and alkaline compounds
- Resistance to osmotic lysis via complement deposition
- Resistance to lethal oxidations and survival inside of macrophages .

Epidemiology

- *M. tuberculosis* infects one third world's population
- Causes 8 million new cases active disease annually, Causes 2 million deaths - 2nd only to HIV as cause of death from infectious agent world wide among adults
- HIV/TB relationship has exacerbated problem with TB increasing in areas with high AIDS incidence- Especially sub-Saharan Africa
- Kenya has a large and rising TB disease burden and is ranked among the twenty-two countries that collectively contribute about 80% of the world's TB cases.
- There were 106,083 in 2010

Epidemiology Cont'

- The most frequent source of infection is the human who excretes, particularly from the respiratory tract, large numbers of tubercle bacilli.
- Risk factors;
  - Exposure to sources of infectious bacilli—principally sputum-positive patients, Rate of active infection in the population, Crowding, Low socioeconomic status, Inadequacy of medical care, Age (high risk in infancy and in the elderly), Undernutrition, Immunologic status, coexisting diseases (eg, silicosis, diabetes), and other individual host resistance factors.

Pathogenesis

- Mycobacteria in droplets 1–5 m in diameter are inhaled and reach alveoli. The disease results from establishment and proliferation of virulent organisms and interactions with the host.
- Resistance and hypersensitivity of the host greatly influence the development of the disease.

Pathology

- The production and development of lesions and their healing or progression are determined by; (1) the number of mycobacteria in the and (2) the resistance and hypersensitivity of the host. **Two Principal Lesions;**

i. **Exudative Type -** consists of an acute inflammatory reaction, with edema fluid, PMN leukocytes, and, later, monocytes around the tubercle bacilli. It may heal by resolution, so that the entire exudate becomes absorbed; it may lead to massive necrosis of tissue; or it may develop into the second (productive) type of lesion.

ii. **Productive Type -** When fully developed, this lesion, a chronic granuloma, consists of three zones: (1) a central area of large, multinucleated giant cells containing tubercle bacilli; (2) a mid zone of pale epithelioid cells, and (3) a peripheral zone of fibroblasts, lymphocytes, and monocytes.

- Later, peripheral fibrous tissue develops, and the central area undergoes caseation necrosis. Such a lesion is called a tubercle. A caseous tubercle may break into a bronchus, empty its contents there, and form a cavity. It may subsequently heal by fibrosis or calcification.

Spread of Organisms in the Host

- Spread by direct extension, through the lymphatic channels and bloodstream, and via the bronchi and gastrointestinal tract.
- In the first infection, tubercle bacilli always spread from the initial site via the lymphatics to the regional lymph nodes.
- The bacilli may spread farther and reach the bloodstream, which in turn distributes bacilli to all organs (miliary distribution). The bloodstream can be invaded also by erosion of a vein by a caseating tubercle or lymph node. If a caseating lesion discharges its contents into a bronchus, they are aspirated and distributed to other parts of the lungs or are swallowed and passed into the stomach and intestines.

Intracellular Site of Growth

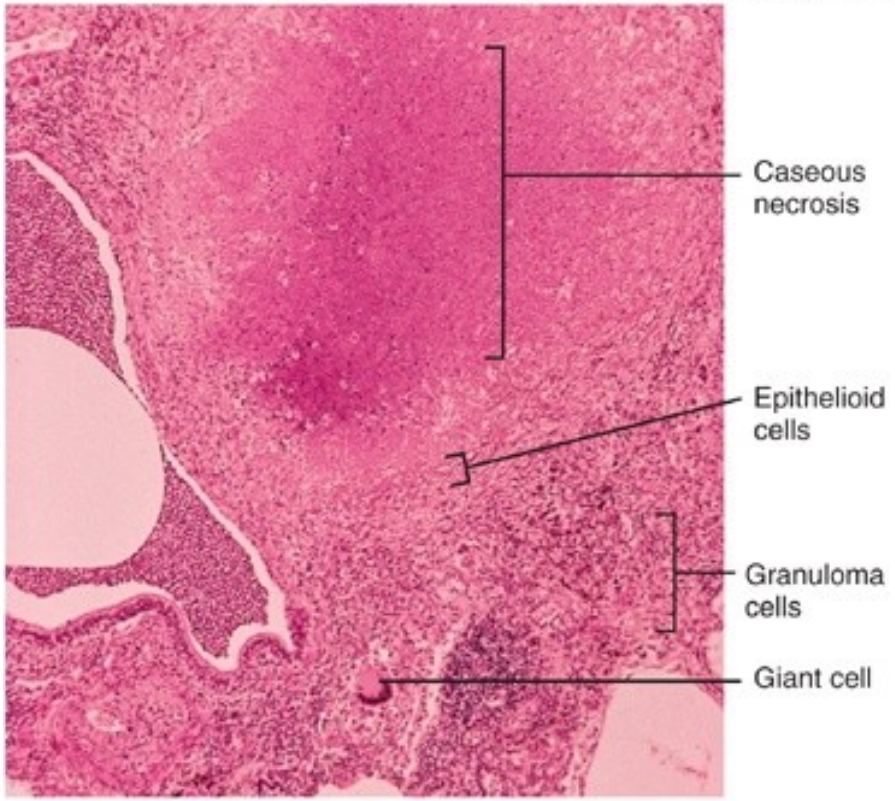
- Once mycobacteria establish themselves in tissue, they reside principally intracellularly in monocytes, reticuloendothelial cells, and giant cells.
- The intracellular location is one of the features that makes chemotherapy difficult and favors microbial persistence.

Course of Infection and Disease

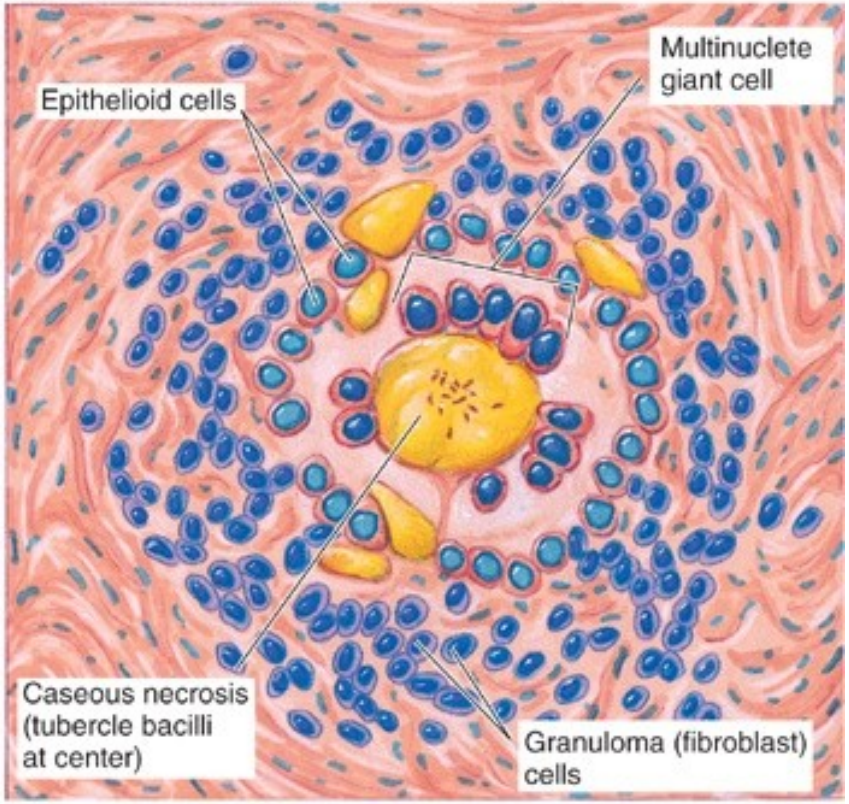
- Only 5% infected people develop clinical disease
- Untreated, the disease progresses slowly; majority of TB cases contained in lungs
- Clinical tuberculosis divided into:
  - Primary tuberculosis
  - Secondary tuberculosis (reactivation or reinfection)
  - Disseminated tuberculosis

Primary TB

- Infectious dose 10 cells
- Phagocytosed by alveolar macrophages and multiply intracellularly
- After 3-4 weeks immune system attacks, forming tubercles, granulomas consisting of a central core containing bacilli surrounded by WBCs – **tubercle**
- If center of tubercle breaks down into necrotic **caseous** lesions, they gradually heal by calcification.



(a) © John D. Cunningham/Visuals Unlimited



(b)

Secondary TB

- If patient doesn't recover from primary tuberculosis, reactivation of bacilli can occur.
- Tubercles expand and drain into the bronchial tubes and upper respiratory tract.
- Gradually the patient experiences more severe symptoms.
  - violent coughing, greenish or bloody sputum, fever, anorexia, weight loss, fatigue
- Untreated, 60% mortality rate

Extrapulmonary TB

- During secondary TB, bacilli disseminate to regional lymph nodes, kidneys, long bones, genital tract, brain, and meninges.
- These complications are grave.

TUBERCULIN TEST

- Old tuberculin is a concentrated filtrate of broth in which tubercle bacilli have grown for 6 weeks. A purified protein derivative (PPD) is obtained by chemical fractionation of old tuberculin.
- **Dose of Tuberculin -** The volume is usually 0.1 mL injected
- **Reactions to Tuberculin**
  - In an individual who has not had contact with mycobacteria, there is no reaction to PPD.
  - An individual who has had a primary infection develops induration, edema, erythema in 24–48 hours, and. The skin test should be read in 48 or 72 hours.
  - Considered positive if the injection of is followed by induration 10 mm or more in diameter. Positive tests tend to persist for several days.
  - The tuberculin test becomes positive within 4–6 weeks after infection (or injection of avirulent bacilli). It may be negative in the presence of tuberculous infection
- **Interpretation of Tuberculin Test**
- A positive tuberculin test indicates that an individual has been infected in the past. It does not imply that active disease or immunity to disease is present.

Diagnostic Laboratory Tests

- A positive tuberculin test does not prove the presence of active disease
- Specimens - fresh sputum, gastric washings, urine, pleural fluid, cerebrospinal fluid, joint fluid, biopsy material, blood, or other suspected material.
- Smears - Sputum, exudates, or other material is examined for acid-fast bacilli by Ziehl-Neelsen staining. Stains of gastric washings and urine generally are not recommended, because saprophytic mycobacteria may be present and yield a positive stain.
- Fluorescence microscopy is more sensitive than acid-fast stain

- The sputum sample for culture is treated with NaOH which kills other contaminating bacteria but not MTB because cells are resistant to alkaline compounds by virtue of their lipid layer.
- Two media are used to grow MTB **- Middlebrook's medium** and Lowenstein-Jensen medium
- Culturing can take 4-6 weeks to yield visible colonies. As a result, another method is commonly used call the **BACTEC System**. Using the BACTEC system, MTB growth can be detected in 9-16 days vs 4-6 weeks using conventional media.

Management and Prevention of TB

- 6-24 months of at least 2 drugs from a list of 11
- One pill regimen called *Rifater* (isoniazid, rifampin, pyrazinamide)
- Vaccine based on attenuated bacilli Calmet-Guerin strain of *M. bovis* used in other countries

Mycobacterium leprae: The Leprosy Bacillus

- Hansen's bacillus/Hansen's Disease
- Strict parasite – has not been grown on artificial media or tissue culture
- Slowest growing of all species
- Multiplies within host cells in large packets called globi
- Causes leprosy, a chronic disease that begins in the skin and mucous membranes and progresses into nerves

Epidemiology and Transmission of Leprosy

- Endemic regions throughout the world
- Spread through direct inoculation from leprotics
- Not highly virulent; appears that health and living conditions influence susceptibility and the course of the disease
- May be associated with specific genetic marker

Course of Infection and Disease

- Macrophages phagocytize the bacilli, but a weakened macrophage or slow T cell response may not kill bacillus.
- Incubation from 2-5 years; if untreated, bacilli grow slowly in the skin macrophages and Schwann cells of peripheral nerves
- 2 forms possible:
  - **tuberculoid** – superficial infection without skin disfigurement which damages nerves and causes loss of pain perception
  - **lepromatous** – a deeply nodular infection that causes severe disfigurement of the face and extremities

Diagnosing

- Combination of symptomology, microscopic examination of lesions, and patient history
- Numbness in hands and feet, loss of heat and cold sensitivity, muscle weakness, thickened earlobes, chronic stuffy nose
- Detection of acid-fast bacilli in skin lesions, nasal discharges, and tissue samples

Treatment and Prevention

- Treatment by long-term combined therapy
- Prevention requires constant surveillance of high risk populations.
- WHO sponsoring a trial vaccine

Infections by Non-Tuberculosis Mycobacteria (NTM)

- *M. avium* complex – third most common cause of death in AIDS patients
- *M. kansaii* – pulmonary infections in adult white males with emphysema or bronchitis
- *M. marinum* – water inhabitant; lesions develop after scraping on swimming pool concrete
- *M. scrofulaceum* – infects cervical lymph nodes
- *M. paratuberculosis* – raw cow's milk; recovered from 65% of individuals diagnosed with Crohn's disease

Actinomycetes: Filamentous Bacilli

- Genera *Actinomyces* & *Nocardia* are nonmotile filamentous bacteria related to mycobacteria.
- May cause chronic infection of skin and soft tissues
- *Actinomyces israelii* – responsible for diseases of the oral cavity, thoracic or intestines - **actinomycoses**
- *Nocardia brasiliensis* causes pulmonary disease similar to TB.

EXOTOXIN

1. Released from the cell before or after lysis
2. Protein
3. Heat labile
4. Antigenic and immunogenic
5. Toxoids can be produced
6. Specific in effect on host
7. Produced by gram-positive and gram-negative organisms

ENDOTOXIN

1. Integral part of cell wall
2. Endotoxin is LPS; Lipid A is toxic component
3. Heat stable
4. Antigenic; ??immunogenicity
5. Toxoids cannot be produced
6. Many effects on host
7. Produced by gram-negative organisms only

ENTEROBACTERIACEAE

IMViC Test

- Indole, Methyl Red, Voges-Prosakaur, Citrate (IMViC) Tests:
  - The tests comprise a series of important determinations that are collectively called the IMViC series of reactions
  - The IMViC series of reactions allows for the differentiation of the various members of *Enterobacteriaceae*.

MEDICALLY IMPORTANT ENTEROBACTERIACEAE

- *Citrobacter* species
- *Enterobacter* spp.
- *Escherichia* spp.
- *Klebsiella* spp.
- *Morganella* spp.
- *Proteus* spp.
- *Salmonella* spp.
- *Serratia* spp.
- *Shigella* spp.
- *Yersinia spp.*

Microbiological Properties

- Gram-negative and rod shaped (bacilli)
- Ferment glucose with acid and (often) gas production
- Reduce nitrate to nitrite
- Grow on 5% sheep blood or chocolate agar in carbon dioxide or ambient air
- Grow anaerobically (facultative anaerobes)
- Catalase positive and cytochrome oxidase negative
- Grow readily on MacConkey (MAC) and eosin methylene blue (EMB) agars
- Grow readily at 35^{o}C except Yersinia (25^{o}-30^{o}C)
- Motile by peritrichous flagella except Shigella and Klebsiella which are non-motile
- Do not form spores

- Somatic O antigens – these are the heat stable polysaccharide part of the LPS.
- Flagellar H antigens – are heat labile
- Envelope or capsule K antigens – overlay the surface O antigen and may block agglutination by O specific antisera.
- Boiling for 15 minutes will destroy the K antigen and unmask O antigens.
- The K antigen is called the **Vi** (virulence) antigen in *Salmonella typhi*.

Antigenic Structure of Enterobacteriaceae

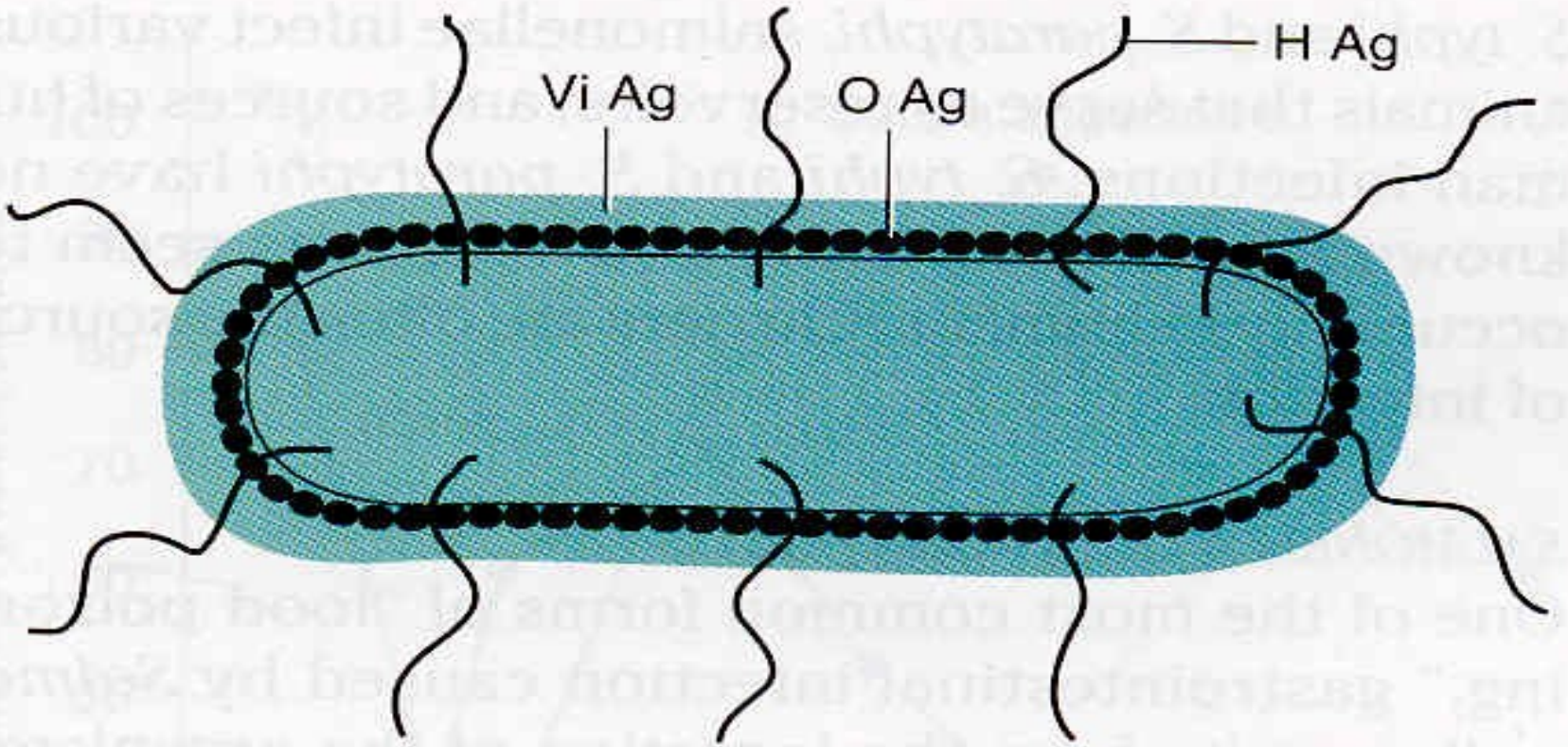


Figure 16-7

The antigenic structures of salmonellae used in serologic typing.

Escherichia coli

- Normal inhabitant of the G.I. tract.
- Some strains cause various forms of gastroenteritis.
- Is a major cause of urinary tract infection and neonatal meningitis and septicemia.
- May have a capsule.
- Most are motile.
- Antigenic structure - has O, H, and K antigens. K1 has a strong association with virulence, particularly meningitis in neonates.

Virulence factors

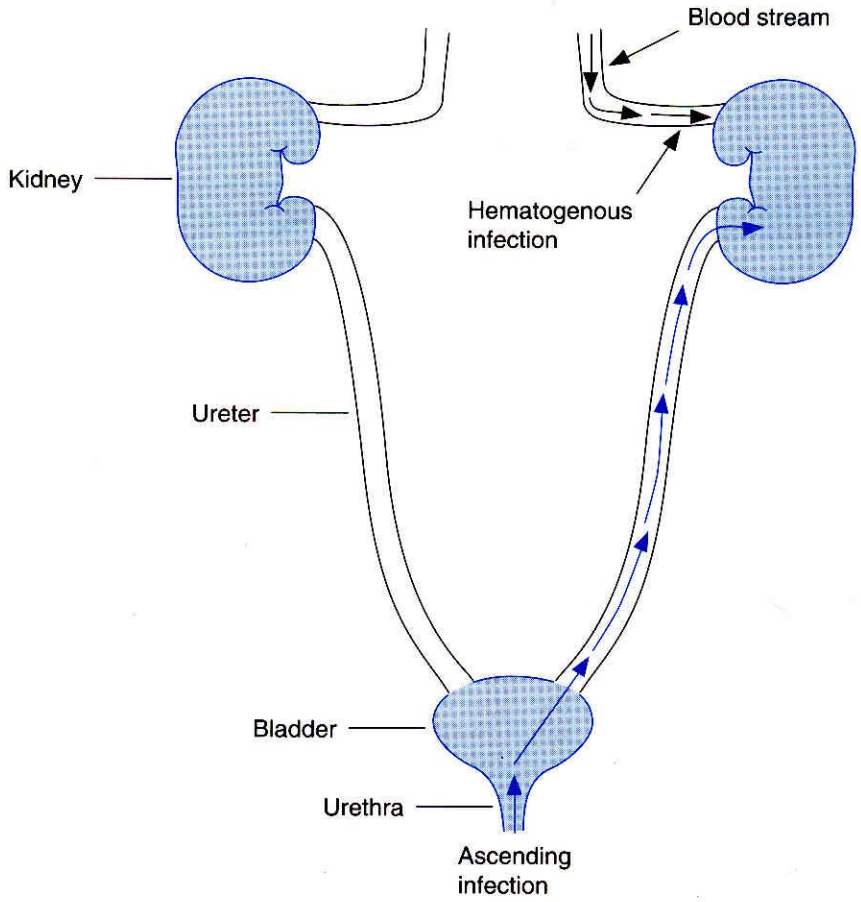
- **Toxins**
  - Enterotoxins – produced by enterotoxigenic strains of *E. coli* (ETEC). Causes a movement of water and ions from the tissues to the bowel resulting in watery diarrhea.
  - Shiga-type toxin – also called the verotoxin -produced by enterohemorrhagic strains of E. coli (EHEC) – is cytotoxic, enterotoxic, neurotoxic, and may cause diarrhea and ulceration of the G.I. tract.
  - Enteroaggregative toxin - (EAEC) – causes watery diarrhea.
  - Hemolysins – two different types may be found: cell bound and secreted.
  - They lyse RBCs and leukocytes and may help to inhibit phagocytosis when cell bound.
  - Endotoxin

- **Adhesions** – are also called colonization factors and include both pili or fimbriae and non-fimbrial factors involved in attachment.
  - Antibodies to these may protect one from colonization.
- Virulence factors that protect the bacteria from host defenses
  - Capsule
  - Outer membrane proteins - are involved in helping the organism to invade by helping in attachment (acting as adhesion) and in initiating endocytosis.

CLINICAL SIGNIFICANCE

- *E coli* is the most common cause of urinary tract infection and accounts for approximately 90% of first urinary tract infections in young women.
- Can lead to acute cystitis (bladder infection) and pyelonephritis (kidney infection).
- S&S - urinary frequency, dysuria, hematuria, and pyuria.
- Urinary tract infection can result in bacteremia with clinical signs of sepsis
- New evidence in women who suffer from recurrent UTIs suggests that this is due to the formation of pod-like *E. coli* biofilms inside bladder epithelial cells.
    - Bacteria living on the edges of the biofilms nay break off leading to a round of infection.

Ascending urinary tract infection



E. coli infections

Neonatal meningitis – is the leading cause of neonatal meningitis and septicemia with a high mortality rate.

- Usually caused by strains with the K capsular antigen.

Gastroenteritis – there are several distinct types of *E. coli* that are involved in different types of gastroenteritis:

- Enterotoxigenic *E. Coli* (ETEC),
- Enteroinvasive *E. Coli* (EIEC),
- Enteropathogenic *E. Coli* (EPEC) ,
- enteroaggregative *E. Coli* (EAEC), and
- Enterohemorrhagic *E. Coli* (EHEC).

E. coli gastroenteritis

- **ETEC** – is a common cause of traveler's diarrhea and diarrhea in children in developing countries.
  - The organism attaches to the intestinal mucosa via colonization factors and then liberates enterotoxin.
  - The disease is characterized by a watery diarrhea, nausea, abdominal cramps and low-grade fever for 1-5 days.
  - Transmission is via contaminated food or water.
- **EPEC** – Diarrhea with large amounts of mucous without blood or pus occurs along with vomiting, malaise and low grade fever.
- This is a problem mainly in hospitalized infants and in day care centers.

E. coli gastroenteritis

- **EIEC** – The organism attaches to the intestinal mucosa via pili and outer membrane proteins are involved in direct penetration, invasion of the intestinal cells, and destruction of the intestinal mucosa.
  - Symptoms include fever, severe abdominal cramps, malaise, and watery diarrhea followed by scanty stools containing blood, mucous, and pus.
- **EAEC** – Mucous associated autoagglutinins cause aggregation of the bacteria at the cell surface and result in the formation of a mucous biofilm.
  - Symptoms include watery diarrhea, vomiting, dehydration and occasional abdominal pain.

E. coli *gastroenteritis*

- **EHEC** – The organism attaches via pili to the intestinal mucosa and liberates the shiga-like toxin.
  - Watery diarrhea progressing to bloody diarrhea and crampy abdominal pain with no fever or low-grade fever.

Antimicrobic therapy

- E. coli is usually susceptible to a variety of chemotherapeutic agents, The sulfonamides, ampicillin, cephalosporins, fluoroquinolones, and aminoglycosides, though drug resistant strains are increasingly prevalent.
  - It is essential to do susceptibility testing.
  - Treatment of patients with EHEC infections is not recommended because it can increase the release of shiga-like toxins and actually trigger HUS

PREVENTION

- HANDWASHING after using the toilet, changing diapers and before preparing or eating food, after contact with animals or their environments
- COOK meats thoroughly.
- AVOID raw milk, unpasteurized dairy products, and unpasteurized juices
- AVOID swallowing water when swimming or playing in lakes, ponds, streams, swimming pools
- PREVENT cross contamination in food preparation areas by thoroughly washing hands, counters, cutting boards, and utensils after they touch raw meat.

Shigella species

- The natural habitat of shigellae is limited to the intestinal tracts of humans and other primates, where they produce bacillary dysentery.
- Contains four species that differ antigenically and, to a lesser extent, biochemically.
  - *S. dysenteriae* (Group A)
  - *S. flexneri* (Group B)
  - *S. boydii* (Group C)
  - *S. sonnei* (Group D)
- All ferment mannitol except *S. dysenteriae*
- *S. sonnei* may show delayed lactose fermentation

- **Antigenic structure**
- Differentiation into groups (A, B, C, and D) is based on O antigen.
- **Virulence factors**
  - Shiga toxin – is produced by *S. dysenteriae* and in smaller amounts by *S. flexneri* and *S. sonnei*. This plays a role in the ulceration of the intestinal mucosa.

Shigellosis - Epidemiology

- Major public health problem in many developing countries
  - causes about 5 to I0% of childhood diarrhoea
  - up to 25% of all diarrhea-related deaths can be associated with Shigella

Developing countries:

- Sh. *flexneri* is endemic
- Sh. dysenteriae type 1 often occurs in an epidemic pattern
- These two species of Shigella generally produce the most severe illness.

Developed countries:

- *Sh. sonnei* is the most common and is the least virulent
- Sh. boydii causes disease of intermediate severity is least common, except in the Indian sub-continent.

Shigellosis - Epidemiology

- Worldwide distribution; infections occur throughout year
- Mostly in children aged under five
- Rates of infection are highest where sanitation is poor
- Transmission influenced by:
  - nutritional status
  - environmental factors affecting transmission:
    - Rainfall and temperature
    - Waterwashed as well as waterborne
- Incidence highest in dryer climates in hot and dry weather
- Incidence in wet climates is often highest in rainy season

Clinical significance

- Causes shigellosis or bacillary dysentery.
- Transmission is via the fecal-oral route.
- The infective dose required to cause infection is very low (10-200 organisms).
- Incubation of 1-7 days followed by fever, cramping, abdominal pain, and watery diarrhea (due to the
- This may be followed by frequent, scant stools with blood, mucous, and pus (due to invasion of intestinal mucosa).
- It is rare for the organism to disseminate.
- The severity of the disease depends upon the species one is infected with.
  - *S. dysenteria* is the most pathogenic followed by *S. flexneri*, *S. sonnei* and *S. boydii*.

Treatment:

- Continue to eat (feed)
  - to prevent weight loss and hypoglycemia
    - include foods rich in potassium (bananas)
- Replace fluids - Oral or IV rehydration with fluids and electrolytes
- Treat with antibiotics:
  - trimethoprim/ sulfamethoxazole, Ciprofloxacin, ampicillin, doxycycline

Prevention and Control:

- Handwashing, especially after defacation
- Improved sanitation and hygiene
- No effective vaccine

Salmonella

- Classification has been changing in the last few years.
- There is now 1 species: *S. enteritica*, and 7 subspecies: 1, 2 ,3a ,3b ,4 ,5, and 6.
- Clinically Salmonella isolates are often still reported out as serogroups or serotypes
- Four serotypes of salmonellae that cause enteric fever can be identified in the clinical laboratory by biochemical and serologic tests. They are;
  - *Salmonella* Paratyphi A (serogroup A),
  - *Salmonella* Paratyphi B (serogroup B),
  - *Salmonella* Choleraesuis (serogroup C1), and
  - *Salmonella* Typhi (serogroup D).

Antigenic Structure

- O antigens - characteristic sequence of repeating polysaccharide units in LPS.
- H antigens - flagellar antigens (protein) and may occur in one of two phase variations.
- Vi antigen - a capsular polysaccharide

Virulence factors

- Endotoxin – may play a role in intracellular survival
- Capsule (for *S. typhi* and some strains of S. *paratyphi*)
- Adhesions – both fimbrial and non-fimbrial

Epidemiology

- Person to-person spread
- No animal reservoir
- Contamination with human faeces
  - Usuallycontaminated water.
  - Occasionally, contaminated food (usually handled by an individual who harbours *S. typhi*)

Carrier states

- Carrier state may last from many weeks to years with faecal shedding
- Convalescent carrier
- Chronic carrier
  - ~3% of persons infected with *S. typhi*
- Potential for cross-contamination of foods by the infected handler - "Typhoid Mary" Mallone

Clinical Significance

- Causes two different kinds of disease: enteric fevers and gastroenteritis.
- Both types of disease begin in the same way, but with the G/E the bacteria remains restricted to the intestine and with the enteric fevers, the organism spreads
- Transmission is via a fecal-oral route
- The organism moves through the intestinal mucosa and adheres to intestinal epithelium.
- For gastroenteritis the *Salmonella* multiply and their presence induces a strong inflammatory response which causes most of the symptoms seen G/E

- In enteric fevers (typhoid and paratyphoid) the Salmonella disseminate before they multiply to high enough levels to stimulate a strong inflammatory
- The bacteria move via the lymphatics and bloodstream to the liver and spleen where phagocytosis and multiplication occurs.
- The bacteria re-enter the bloodstream to disseminate throughout the body to all organs causing fever, headaches, myalgia, and GI problems.
- Rose spots (erythematous, muculopapular lesions) are seen on the abdomen. Osteomyelitis, cystitis, and gall bladder infections may occur.

Diagnosis of typhoid fever

- Blood cultures are positive during the first week and after the second week
- Stool cultures and sometimes urine cultures are positive after the second week
- The Widal test is a serological test for antibodies against *Salmonella typhi*. One looks for a 4-fold rise in titer between acute and convalescent stages.
- 10% of those infected become short term carriers and a smaller % become long-term carriers due to persistence of the bacteria in the gallbladder or urinary bladder.

- Differential Medium Cultures - EMB, MacConkey's, or deoxycholate medium permits rapid detection of lactose nonfermenters Many salmonellae produce H_2S.
- Selective Medium Cultures - The specimen is plated on salmonella-shigella (SS) agar, Hektoen enteric agar, XLD, or deoxycholate-citrate agar, which favor growth of salmonellae and shigellae over other Enterobacteriaceae.
- Enrichment Cultures - The specimen (usually stool) also is put into selenite F or tetrathionate broth, both of which inhibit replication of normal intestinal bacteria and permit multiplication of salmonellae. After incubation for 1–2 days, this is plated on differential and selective media.
- Serologic Methods
- Agglutination Test - known sera and unknown culture are mixed on a slide. Clumping, when it occurs, can be observed within a few minutes.

- Tube Dilution Agglutination Test (Widal Test) - Serum agglutinins rise sharply during the second and third weeks of *Salmonella* Typhi infection. The Widal test to detect these antibodies against the O and H antigens has been in use for decades. At least two serum specimens, obtained at intervals of 7–10 days, are needed to prove a rise in antibody titer.
- Serial dilutions of unknown sera are tested against antigens from representative salmonellae.
- The interpretive criteria when single serum specimens are tested vary, but a titer against the O antigen of > 1:320 and against the H antigen of > 1:640 is considered positive.
- High titer of antibody to the Vi antigen occurs in some carriers. Results of serologic tests for salmonella infection must be interpreted cautiously because the possible presence of cross-reactive antibodies limits the use of serology. The test is not useful in diagnosis of enteric fevers caused by salmonella other than *Salmonella* Typhi.

TREATMENT

- **Gastroenteritis:** Usually a self-limiting disease. Fluid and electrolyte replacement.
- Recommended oral regimens for the treatment of **typhoid fever :**
  - Fluoroquinolone
  - Alternatively, use of an intravenous antibiotic, such as ceftriaxone, is effective in patients who cannot tolerate oral medications.

Prevention

1. Remove source
   - Salmonella free life-stock
   - Vaccinate chicks
2. Interrupt transmission

Good food hygiene

- Cook food properly
- Keep raw and cooked foods apart

Public Health: clean water

3. Strengthen host
   - Vaccination

Yersinia species

- Are short, pleomorphic gram-negative rods that can exhibit bipolar staining.
- Catalase-positive, oxidase-negative, and microaerophilic or facultatively anaerobic.
- Most have animals as their natural hosts, but they can produce serious disease in humans.
- Includes;
  - *Yersinia pestis,* the cause of plague;
  - *Yersinia pseudotuberculosis* and *Yersinia enterocolitica,* important causes of human diarrheal diseases; and others.

Identification

- *Y. pestis* can be separated from *Y. enterocolitica* and *Y. pseudotuberculosis* by the fact that it is non-motile.
- *Y. enterocolitica* and *Y. pseudotuberculosis* are both non-motile at 37^0 C, and motile at 22^0 C.
- *Y. pestis* is identified based on the following:
  - Non-motile
  - Bipolar staining
  - Slow growth of small colonies on ordinary culture media – it grows better at lower temperature (25-30^0 C)

Virulence characteristics

- Endotoxin – is responsible for many of the symptoms
- Murine toxin – causes edema and necrosis in mice and rats, but has not been shown to play a role in human disease
- V antigen – a secreted protein that controls expression of many of the virulence factors

Pathogenesis & Pathology

- When a flea feeds on a rodent infected with *Y pestis*, the ingested organisms multiply in the gut of the flea and, helped by the coagulase, block its proventriculus so that no food can pass through. Subsequently, the "blocked" and hungry flea bites ferociously and the aspirated blood, contaminated with *Y pestis* from the flea, is regurgitated into the bite wound.

Pathogenesis & Pathology

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- Inoculated organisms phagocytosed by PMN cells and monocytes. The *Y pestis* organisms are killed by the polymorphonuclear cells but multiply in the monocytes

Pathogenesis & Pathology

- Pathogens rapidly reach the lymphatics and an intense hemorrhagic inflammation develops in the enlarged lymph nodes, which may undergo necrosis.
- Reach the bloodstream and become widely disseminated.
- Hemorrhagic and necrotic lesions may develop in all organs; meningitis, pneumonia, are prominent features.
- Primary pneumonic plague results from inhalation of infective droplets (usually from a coughing patient), with hemorrhagic consolidation, sepsis, and death.

Clinical significance

- In man plague occurs in two forms; bubonic and pneumonic

Bubonic plague – transmitted by fleas from an infected rodent

- The bacteria travel in the blood to the nearest lymph node where they are engulfed by fixed macrophages.
- A high fever develops and the lymph nodes in the groin and armpit become enlarged (buboes) as the bacteria proliferate and stimulate an inflammatory response.
- The bacteria growing in the lymph node leak into the bloodstream.
- Lysis of the bacteria releases LPS, causing septic shock.
- Subcutaneous hemorrhages, gave the disease the name, the black death, in the middle ages.

Buboes

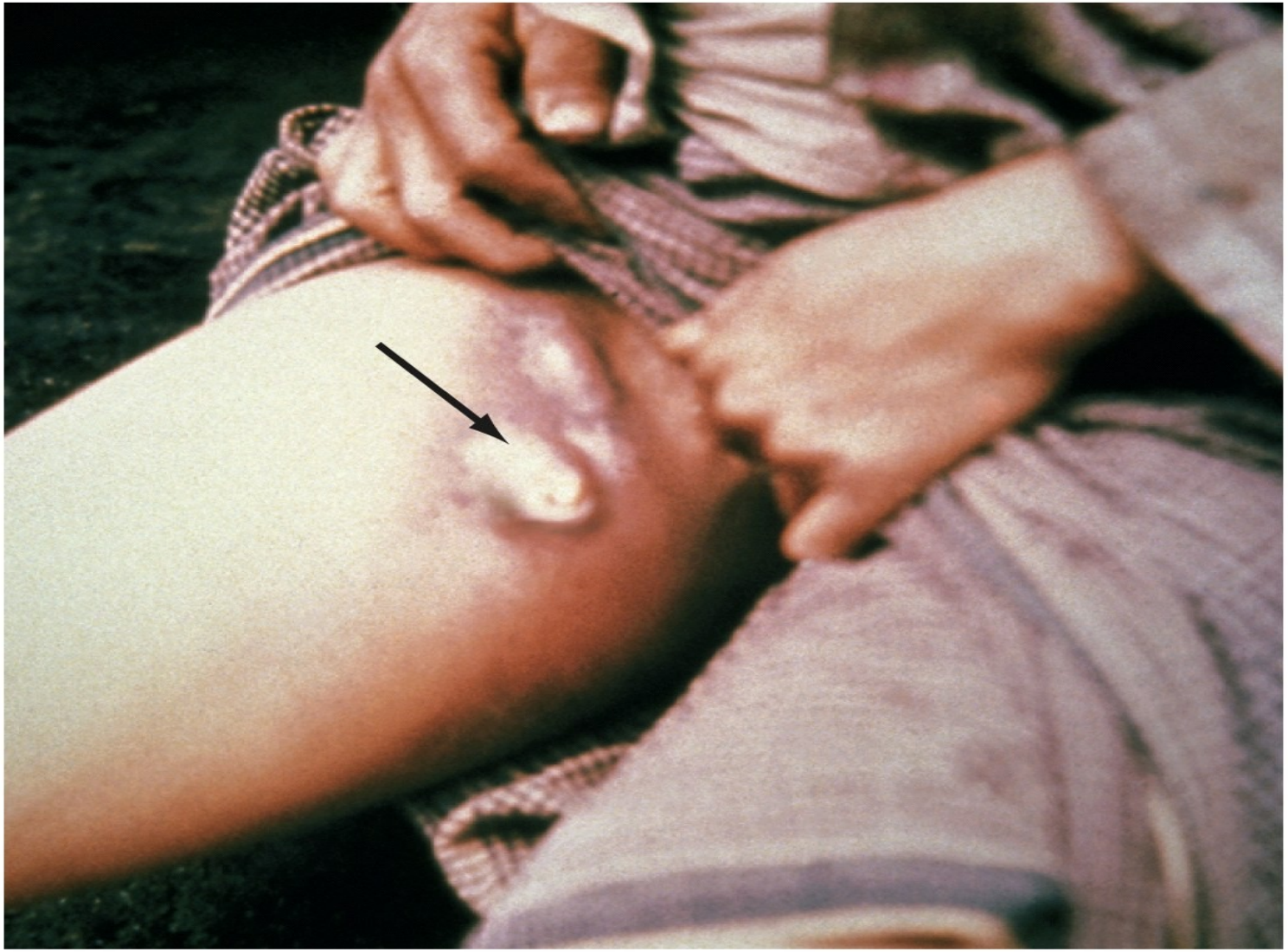

Figure 26.24a Microbiology: An Evolving Science
CDC

- Eventually bacteria reach the lungs where they are ingested by lung macrophages to cause pneumonic plague.

Pneumonic plague – this can be transmitted directly to others via aerosol.

- Direct inhalation of aerosols containing the organism produces a form of the disease that progresses much more rapidly and the mortality rate is close to 100%.

Diagnostic Laboratory Tests

- **Specimens** – Blood for culture and aspirates of enlarged lymph nodes for smear and culture, in possible meningitis CSF is taken
- **Smears** - Giemsa's stain and specific immunofluorescent stains. With Wayson's stain, *Y pestis* shows a striking bipolar appearance.
- **Culture -** All materials are cultured on blood agar and MacConkey's agar plates and in infusion broth.
- Definite identification of cultures is done by immunofluorescence
- Cultures are highly infectious and must be handled with extreme caution.
- **Serology -** serum antibody titer of 1:16 or greater is presumptive evidence of *Y pestis* infection. A titer rise in two sequential specimens confirms the serologic diagnosis.

Treatment

- Unless promptly treated, plague may have a mortality rate of nearly 50%; pneumonic plague, nearly 100%. The drug of choice is streptomycin. Tetracycline is an alternative drug and is sometimes given in combination with streptomycin. Drug resistance has been noted in *Y pestis*.

Epidemiology & Control

- Plague is an infection of wild rodents that occurs in many parts of the world. Epizootics with high mortality rates occur intermittently; at such times, the infection can spread to domestic rodents (eg, rats) and other animals (eg, cats), and humans can be infected by flea bites or by contact.
- The commonest vector of plague is the rat flea *(Xenopsylla cheopis)*, but other fleas may also transmit the infection.

- **Control;**
  - By destruction of plague-infected animals.
  - All patients with suspected plague should be isolated
  - All specimens must be treated with extreme caution.
  - Contacts of patients with suspected plague pneumonia should receive tetracycline, as chemoprophylaxis.
  - A formalin-killed vaccine is available for travelers to hyperendemic areas and for persons at special high risk.

KLEBSIELLA-ENTEROBACTER SERRATIA GROUP

Important Properties

- Frequently found in the large intestine but are also present in soil and water
- Group has very similar properties and are usually disintiguished on the basis of biochemical reactions and motility
- ***Klebsiella pneumoniae*** has a very large capsule (giving the colonies striking mucoid appearance)
- ***Serratia marcescens*** produces red pigmented colonies

Pathogenesis and Epidemiology

A. *Klebsiella pneumoniae*

- Most likely to be a primary non-opportunistic pathogen (related to its antiphagocytic capsule)
- **Predisposing factors;**
  - advanced age
  - chronic respiratory disease
  - diabetes
  - alcoholism
  - 10% of healthy people are carriers

B. Enterobacter /Serratia

- Associated with hospital acquired infections sec to invasive procedures, intravenous catheterization, respiratory intubation, urinary tract manipulation,
- Outbreaks of Serratia pnuemonia have been associated with contamination of the water in respiratory therapy devices
- **Clinical Findings/Disease**
- UTI
- Bacteremiaà sepsis
- Meningitis
- Pneumonia - if due to Klebsiella, patient produces thick, bloody sputum and can progress to necrosis and abscess formation

Laboratory Diagnosis

- Lactose fermenting colonies on differential agar (MacConkey's or EMB)

Treatment

- Frequently resistant to multiple antibiotics especially if hospital acquired
- Empiric treatment with cephalosporin plus an aminoglycosides pending result of sensitivity testing

Prevention

- Changing site of intravenous catheters
- Removing urinary catheters when they are no longer required

Genus : Proteus

- The Genus is characterized by:
  - rapid urease activity (within 4 hours).
  - typical swarming growth on nutrient and blood agar plates.

- The organisms are found in soil, water and fecally contaminated materials.

- Clinically important species include:

1. ***Proteus mirabilis***
   - Isolated from wound and urinary tract infections.
   - Indole negative.
   - Susceptible to ampicillin and cephalosporins.
2. ***Proteus vulgaris***

- Causes urinary tract infection (often nosocomial, affect immunosuppressed patients and those receiving prolonged antibiotic therapy).
- Indole positive.
- Resistance to both ampicillin and cephalosporins.

Laboratory diagnosis

- Specimen: urine, pus, ……etc
- Microscopy: Gram-negative rods.
- Culture: on blood agar medium - swarming growth.

  ☛ on MacConkey's agar → non-lactose fermenting colonies.

- Identification: sugar fermentation – rapid urease activity.

Treatment

According to culture & antibiotic sensitivity test results.

Citrobacter

- Commonly associated with hospital – acquired infections
- Are opportunistic pathogens causing urinary tract or respiratory tract infections and occasionally wound infections, osteomyelitis, endocarditis, and meningitis.
- Especially in immunocompromised patients.

Treatment

- According to culture and antibiotic sensitivity tests results.

Enterobacter

- Clinical significance
  - Nosocomial infections
  - Bacteremia in burn patients

Serratia

- A free-living saprophyte
- Motility
- Has been found in RT and UT infections
- Is resistant to many antimicrobials

VIBRIO, CAMPYLOBACTER, AND *HELICOBACTER*

- Are gram-negative rods that are all widely distributed in nature.
- The vibrios are found in marine and surface waters.
- The campylobacters are found in many species of animals, including many domesticated animals. *Campylobacter jejuni* is a common cause of enteritis in humans.
- *Vibrio cholerae* produces an enterotoxin that causes cholera, a profuse watery diarrhea that can rapidly lead to dehydration and death.
- *Helicobacter pylori* has been associated with gastritis and duodenal ulcer disease.

GENUS *VIBRIO*

- Consists of Gram-negative straight or curved rods, motile by means of a single polar flagellum.
- Most species are oxidase-positive.
- In most ways vibrios are related to enteric bacteria
- Vibrios are distinguished from enterics by being oxidase-positive and motile by means of polar flagella.
- Of the vibrios that are clinically significant to humans, *Vibrio cholerae,* the agent of cholera, is the most important.

- Most vibrios have simple growth factor requirements and will grow in synthetic media with glucose as a sole source of carbon and energy. However, since vibrios are typically marine organisms, most species require 2-3% NaCl or a sea water base for optimal growth.
- Vibrios are one of the most common organisms in surface waters of the world. They occur in both marine and freshwater habitats and in associations with aquatic animals. Some species are bioluminescent and live in mutualistic associations with fish and other marine life.
- Other species are pathogenic for fish, eels, and frogs, as well as other vertebrates and invertebrates.

Antigenic Structure

- Many vibrios share a single heat-labile flagellar H antigen.
- *V cholerae* has O lipopolysaccharides that confer serologic specificity.
- The serotypes are Ogawa, Inaba, and Hikojima. Two biotypes of epidemic *V cholerae* have been defined, classic and El Tor.

Vibrio cholerae Enterotoxin

- *V cholerae* produce a heat-labile enterotoxin which leads to prolonged hypersecretion of water and electrolytes.

Epidemiology

- Six pandemics (worldwide epidemics) of cholera occurred between 1817 and 1923, largely originating in Asia, usually the Indian subcontinent.
- The seventh pandemic began in 1961 in the Indonesia, with spread to Asia, the Middle East, and Africa.
- Cholera is endemic in India and Southeast Asia. From these centers, it is carried along shipping lanes, trade routes, and pilgrim migration routes.
- The disease is spread by contact involving individuals with mild or early illness and by water, food, and flies.
- Vibrios survive in water for up to 3 weeks. *Vibrio cholerae* lives in aquatic environments. And such environments are the vibrios natural reservoir.

Pathogenesis

- A person with normal gastric acidity may have to ingest as many as 10^{10} or more *V cholerae* to become infected when the vehicle is water, because the organisms are susceptible to acid.
- When the vehicle is food, as few as 10^{2}–10^{4} organisms are necessary because of the buffering capacity of food. Any medication or condition that decreases stomach acidity makes a person more susceptible to infection with *V cholerae*.
- Cholera is not an invasive infection. The organisms do not reach the bloodstream but remain within the intestinal tract where they multiply and liberate cholera toxin

Cholera

- **Cholera** is a severe diarrheal disease caused by the bacterium ***Vibrio cholerae*.**
- Transmission is by water or food.
- *V. cholerae* produces **cholera toxin**, whose action on the mucosal epithelium is responsible for the characteristic diarrhea of the disease cholera.
- Cholera is one of the most rapidly fatal illnesses known. A healthy person may become hypotensive within an hour of the onset of symptoms and may die within 2-3 hours if no treatment is provided.
- More commonly, the disease progresses from the first liquid stool to shock in 4-12 hours, with death following in 18 hours to several days.

Clinical Findings

- About 60% of infections with classic *V cholerae* are asymptomatic, as are about 75% of infections with the El Tor biotype.
- Incubation period is 1–4 days
- Sudden onset of nausea and vomiting and profuse diarrhea with abdominal cramps. Stools, which resemble "rice water," contain mucus, epithelial cells, and large numbers of vibrios.
- Rapid loss of fluid and electrolytes, which leads to profound dehydration, circulatory collapse, acidosis and anuria.
- Loss of fluid due to cholera enterotoxin activating the adenylate cyclase enzyme in the intestinal cells, converting them into pumps which extract water and electrolytes from blood and tissues and pump it into the lumen of the intestine.
- The mortality rate without treatment is between 25% and 50%.

Diagnostic Laboratory Tests

- **Specimens** - mucus flecks from stools.
- **Smears -** The microscopic appearance of smears made from stool samples is not distinctive. Dark-field or phase contrast microscopy may show the rapidly motile vibrios.
- **Culture**
- Growth is rapid in peptone agar, on blood agar with a pH near 9.0, or on TCBS (**thiosulfate-citrate-bile-sucrose**) agar, and typical colonies can be picked in 18 hours. For enrichment, a few drops of stool can be incubated for 6–8 hours in taurocholate-peptone broth (pH 8.0–9.0)
- **Specific Tests -** slide agglutination tests using anti-O group 1 or group 139 antisera and by biochemical reaction patterns.

Treatment

- The most important part of therapy consists of water and electrolyte replacement to correct the severe dehydration and salt depletion.
- Most antibiotics and chemotherapeutic agents have no value in cholera therapy.
- However oral tetracycline tends to reduce stool output in cholera and shortens the period of excretion of vibrios.

Control

- Control rests on education and on improvement of sanitation, particularly of food and water.
- Patients should be isolated, their excreta disinfected, and contacts followed up.
- Chemoprophylaxis with antimicrobial drugs may have a place.
- Repeated injection of a vaccine containing either lipopolysaccharides extracted from vibrios or dense vibrio suspensions can confer limited protection to heavily exposed persons (eg, family contacts) but is not effective as an epidemic control measure.

Campylobacter jejuni & *Campylobacter coli*

- *Campylobacter jejuni* and *Campylobacter coli* have emerged as common human pathogens, causing mainly enteritis and occasionally systemic infection.
- Cause infections that are clinically indistinguishable
- These bacteria are at least as common as salmonellae and shigellae as a cause of diarrhea
- Are gram-negative rods with comma, S,
- Motile, with a single polar flagellum, and do not form spores.

Toxins

- Have lipopolysaccharides with endotoxic activity.
- Cytopathic extracellular toxins and enterotoxins have been found

Pathogenesis & Pathology

- The infection is via oral route from food, drink, or contact with infected animals or animal products.
- The organisms multiply in the small intestine, invade the epithelium, and produce inflammation that results in the appearance of red and white blood cells in the stools.
- Occasionally, the bloodstream is invaded and a clinical picture of enteric fever develops.

Clinical Findings

- Acute onset of crampy abdominal pain, profuse diarrhea that may be grossly bloody, headache, malaise, and fever.
- Usually the illness is self-limited to a period of 5–8 days, but occasionally it continues longer.
- *C jejuni* isolates are usually susceptible to erythromycin, and therapy shortens the duration of fecal shedding of bacteria. Most cases resolve without antimicrobial therapy.

Diagnostic Laboratory Tests

- **Specimens -** Diarrheal stool is the usual specimen.
- **Smears -** Gram-stained smears of stool may show the typical shaped rods. Dark-field or phase contrast microscopy may show the typical darting motility of the organisms.
- **Culture -** Culture on the selective media with O_2 (5% O_2) with added CO_2 (10%) is the definitive test to diagnose *C jejuni* enteritis

Helicobacter pylori

- It is a spiral-shaped gram-negative rod.
- *H pylori* is associated with antral gastritis, duodenal (peptic) ulcer disease, gastric ulcers, and gastric carcinoma.
- It has multiple flagella at one pole and is actively motile.

Pathogenesis & Pathology

- Grows optimally at a pH of 6.0–7.0 and would be killed or not grow at the pH within the gastric lumen. Gastric mucus is relatively impermeable to acid and has a strong buffering capacity. On the lumen side of the mucus, the pH is low (1.0–2.0) while on the epithelial side the pH is about 7.4. *H pylori* is found deep in the mucous layer near the epithelial surface where physiologic pH is present

Pathogenesis & Pathology Cont'

- Produces a protease that modifies the gastric mucus and further reduces the ability of acid to diffuse through the mucus.
- Produces urease activity, which yields ammonia and further buffering of acid.
- There is a strong association between the presence of *H pylori* infection and duodenal ulceration.
- Histologically, gastritis is characterized by chronic and active inflammation.
- *H pylori* may be a major risk factor for gastric cancer.

Clinical Findings

- Acute infection can yield an upper gastrointestinal illness with nausea and pain; vomiting and fever may be present also. The acute symptoms may last for less than 1 week or as long as 2 weeks.
- Once colonized, the *H pylori* infection persists for years and perhaps decades or even a lifetime.
- About 90% of patients with duodenal ulcers and 50–80% of those with gastric ulcers have *H pylori* infection.
- *H pylori* also may have a role in gastric carcinoma and lymphoma.

Diagnostic Laboratory Tests

- **Specimens** - Gastric biopsy specimens, Blood
- **Smears -** The diagnosis of gastritis and *H pylori* infection can be made histologically, Giemsa or special silver stains can show the curved or spiraled organisms.
- **Antibodies -** Several assays have been developed to detect serum antibodies specific for *H pylori.*
- **Special Tests -** Rapid tests to detect urease activity are widely used for presumptive identification of *H pylori* in specimens.

Treatment

- Triple therapy with metronidazole and either bismuth subsalicylate or bismuth subcitrate plus either amoxicillin or tetracycline for 14 days eradicates *H pylori* infection in 70–95% of patients.
- An acid-suppressing agent given for 4–6 weeks enhances ulcer healing. Proton pump inhibitors directly inhibit *H pylori* and appear to be potent urease inhibitors.
- Either 1 week of a proton pump inhibitor plus amoxicillin and clarithromycin or of amoxicillin plus metronidazole also is highly effective.

Epidemiology & Control

- *H pylori* is present on the gastric mucosa of less than 20% of persons under age 30 but increases in prevalence to 40–60% of persons age 60, including persons who are asymptomatic.
- In developing countries, the prevalence of infection may be 80% or higher in adults.
- Person-to-person transmission of *H pylori* is likely

SPIROCHETES

- The spirochetes are a large, heterogeneous group of spiral, motile bacteria.
- The family Treponemataceae includes three genera of medical importance: Treponema, Borrelia, and Leptospira.
- They are long, slender, helically coiled, spiral or corkscrew-shaped, gram-negative bacilli.
- Treponemes reproduce by transverse fission.
- Axial filaments - (a form of flagella) - are the locomotory organelles
- Cannot be cultured in vitro

Histopathology showing *Treponema pallidum* spirochetes in testis of experimentally infected rabbit. Modified Steiner silver stain.

jimmy ongori 2013

C... Dr. Edwin P. Ewing, Jr. epe1@cdc.gov

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TREPONEMA PALLIDUM

- Slender spirals measuring about 0.2 m in width and 5–15 m in length.
- The spiral coils are regularly spaced at a distance of 1 m from one another. The organisms are actively motile, rotating steadily around their endoflagella even after attaching to cells by their tapered ends.
- The spirals are so thin that they are not readily seen unless immunofluorescent stain or darkfield illumination is employed.

Epidemiology

- Transmission: sexual contact (overwhelming majority), kissing or other close contact with lesion, transfusion, or direct inoculation
- Most infectious early in disease: chancre, mucous patch, condyloma latum
- Blood transfusion rare: all donors tested and organism cannot survive longer than 48 hrs using current blood bank storage techniques
- Congenital infection
- 12 million cases worldwide

Culture

- Pathogenic *T pallidum* has never been cultured continuously on artificial media, in fertile eggs, or in tissue culture.
- *T pallidum* is a microaerophilic organism; it survives best in 1–4% oxygen.

Reactions to Physical and Chemical Agents

- Drying kills the spirochete rapidly, as does elevation of the temperature to 42 °C.
- Killed by trivalent arsenical, mercury, and bismuth
- Penicillin is treponemicidal in minute concentrations, but the rate of killing is slow, of slow multiplication rate of *T pallidum* (estimated division time is 30 hours).
- Resistance to penicillin has not been demonstrated in syphilis

Virulence Factors of *T. pallidum*

- Outer membrane proteins promote adherence
- Hyaluronidase may facilitate perivascular infiltration
- Antiphagocytic coating of fibronectin
- Tissue destruction and lesions are primarily result of host's immune response (immunopathology)

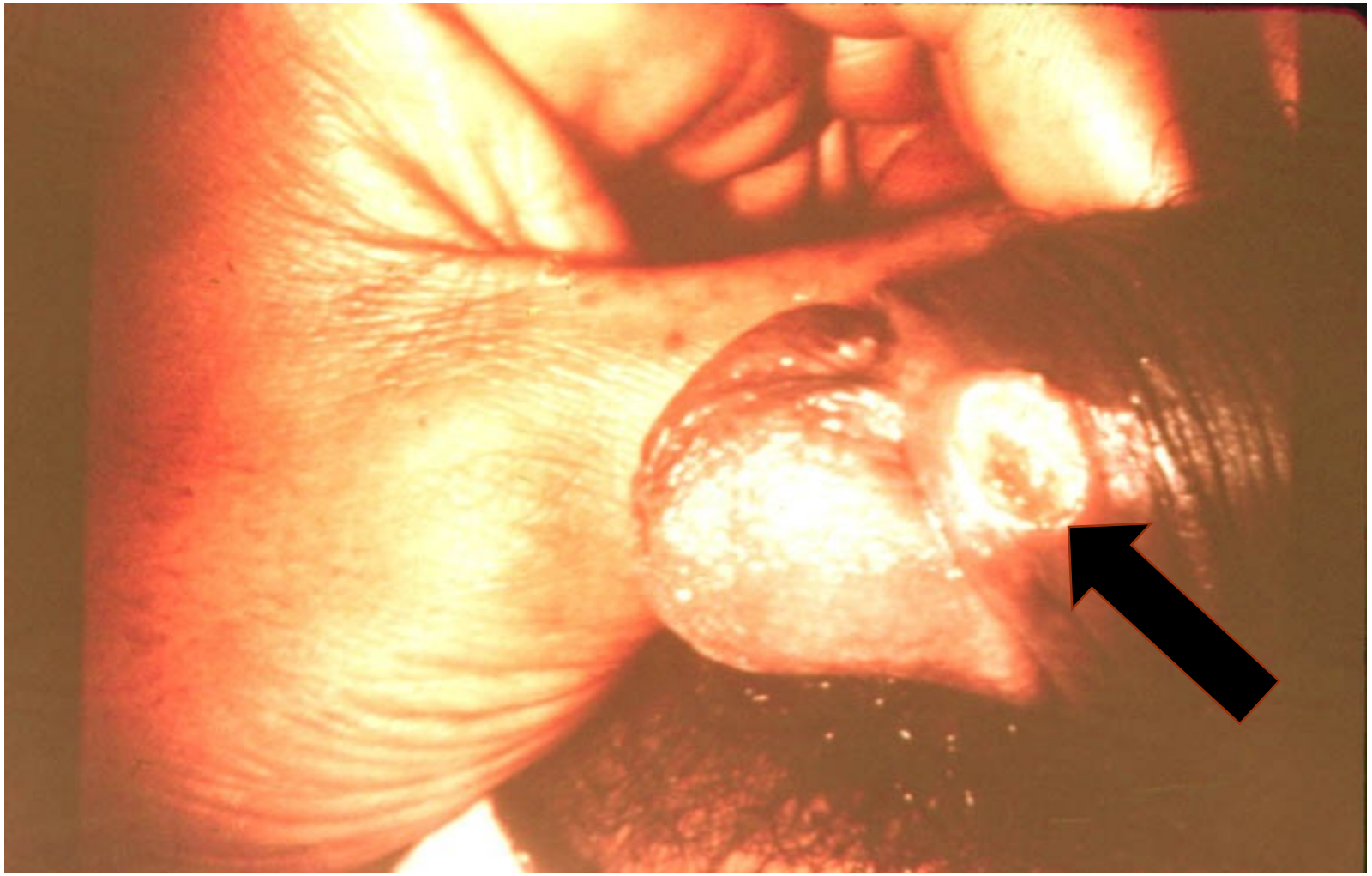
Pathogenesis of T. pallidum

- Tissue destruction and lesions are primarily a consequence of patient's immune response
- Syphilis is a disease of blood vessels and of the perivascular areas
  - In spite of a vigorous host immune response the organisms are capable of persisting for decades
  - Infection is neither fully controlled nor eradicated
  - In early stages, there is an inhibition of cell-mediated immunity
  - Inhibition of CMI abates in late stages of disease, hence late lesions tend to be localized

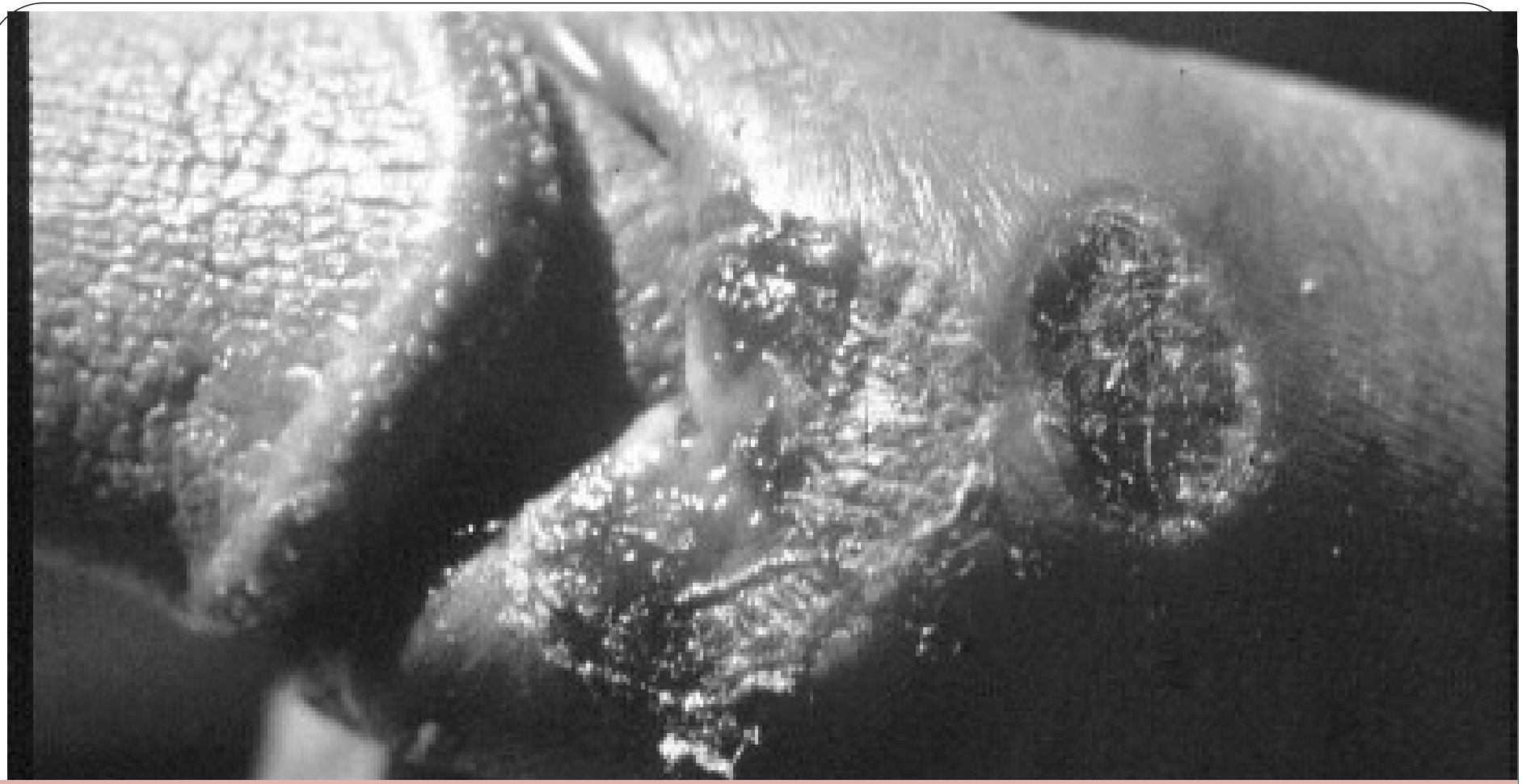
Primary Syphilis

- 1^0 disease process involves invasion of mucus membranes, rapid multiplication & wide dissemination through perivascular lymphatics and systemic circulation - Occurs prior to development of the primary lesion
- 10-90 days after initial contact the host mounts an inflammatory response at the site of inoculation resulting in the hallmark syphilitic lesion, called the **chancre** (usually painless)
- Chancre changes from hard to ulcerative with profuse shedding of spirochetes

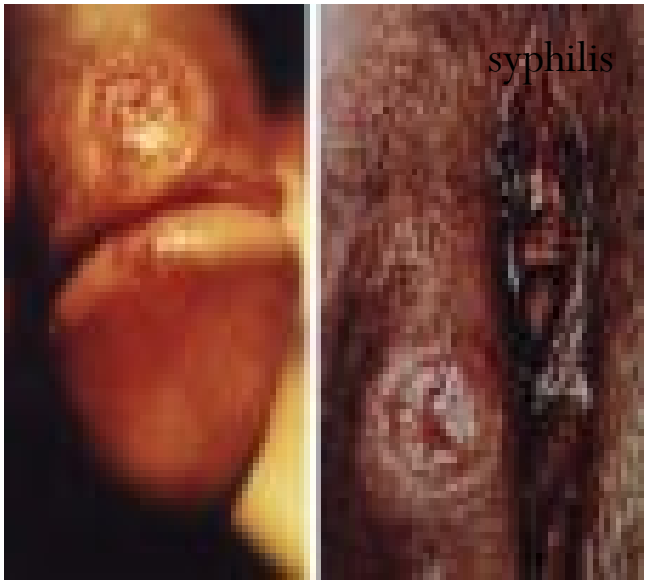
After initial infection, A **primary chancre** is seen in the area of contact within **10-60 days**.

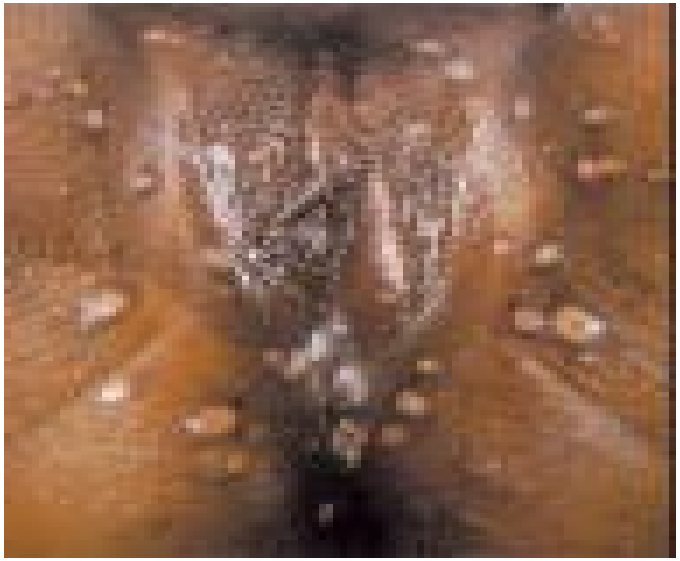


(a primary chancre is an **area of ulceration/inflammation**)

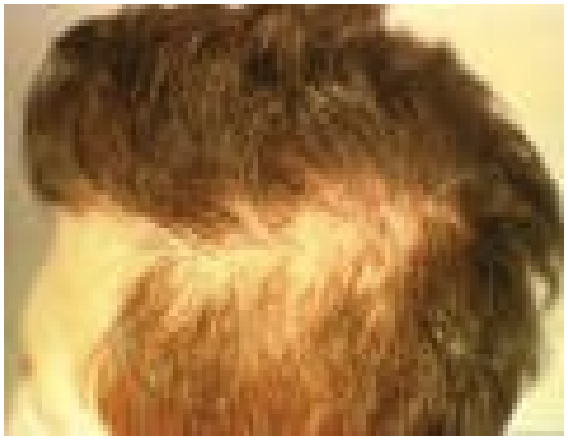


CHANCRE

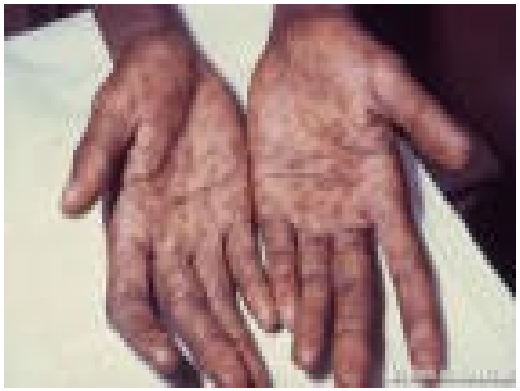


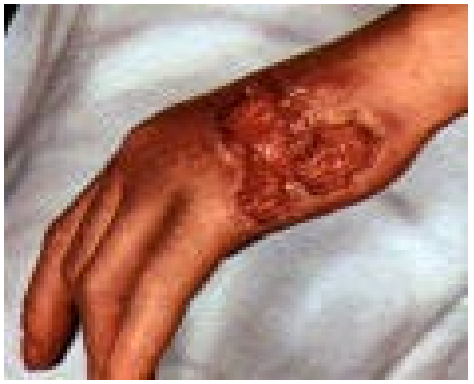


Treponema pallidum

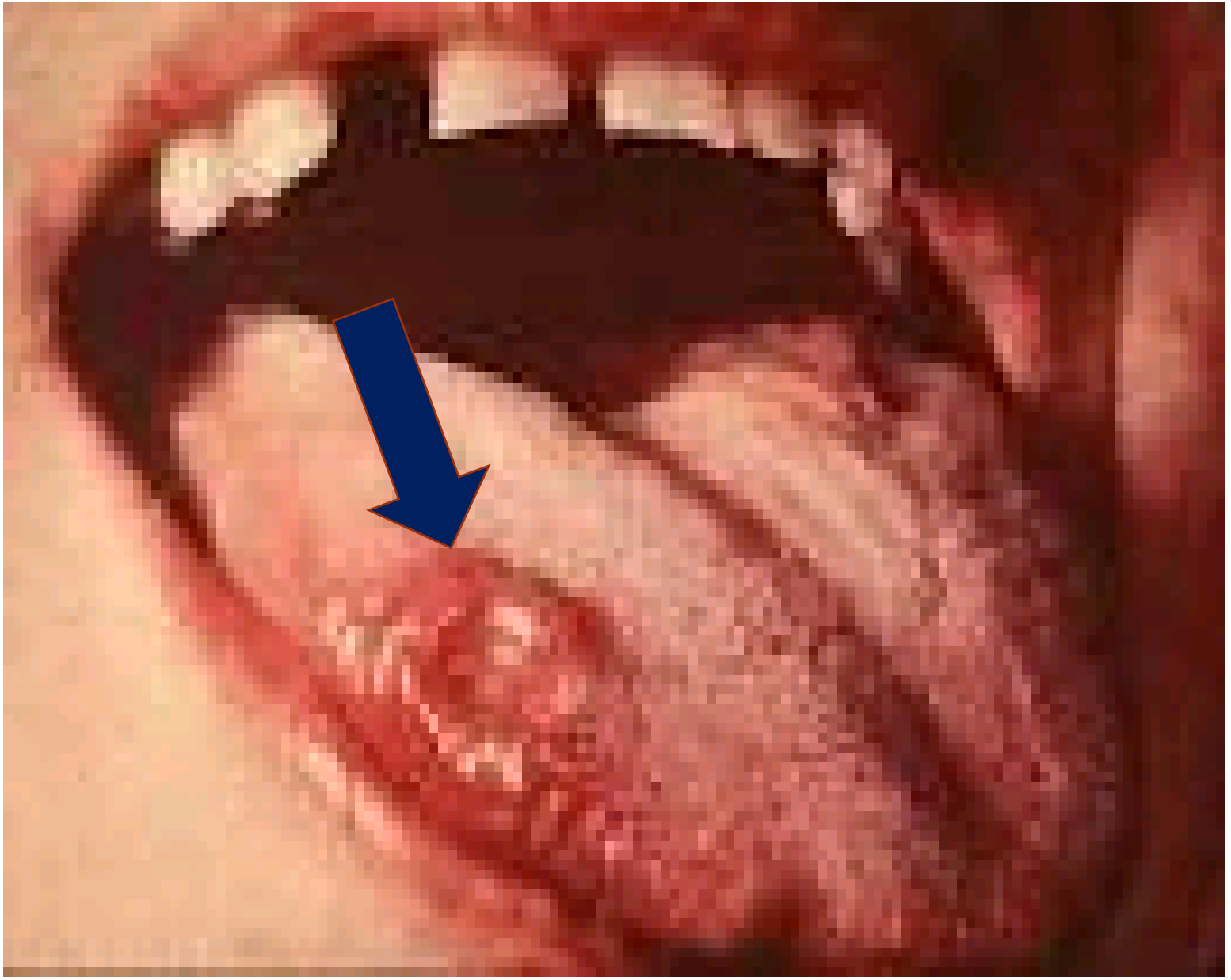


rash





gumma



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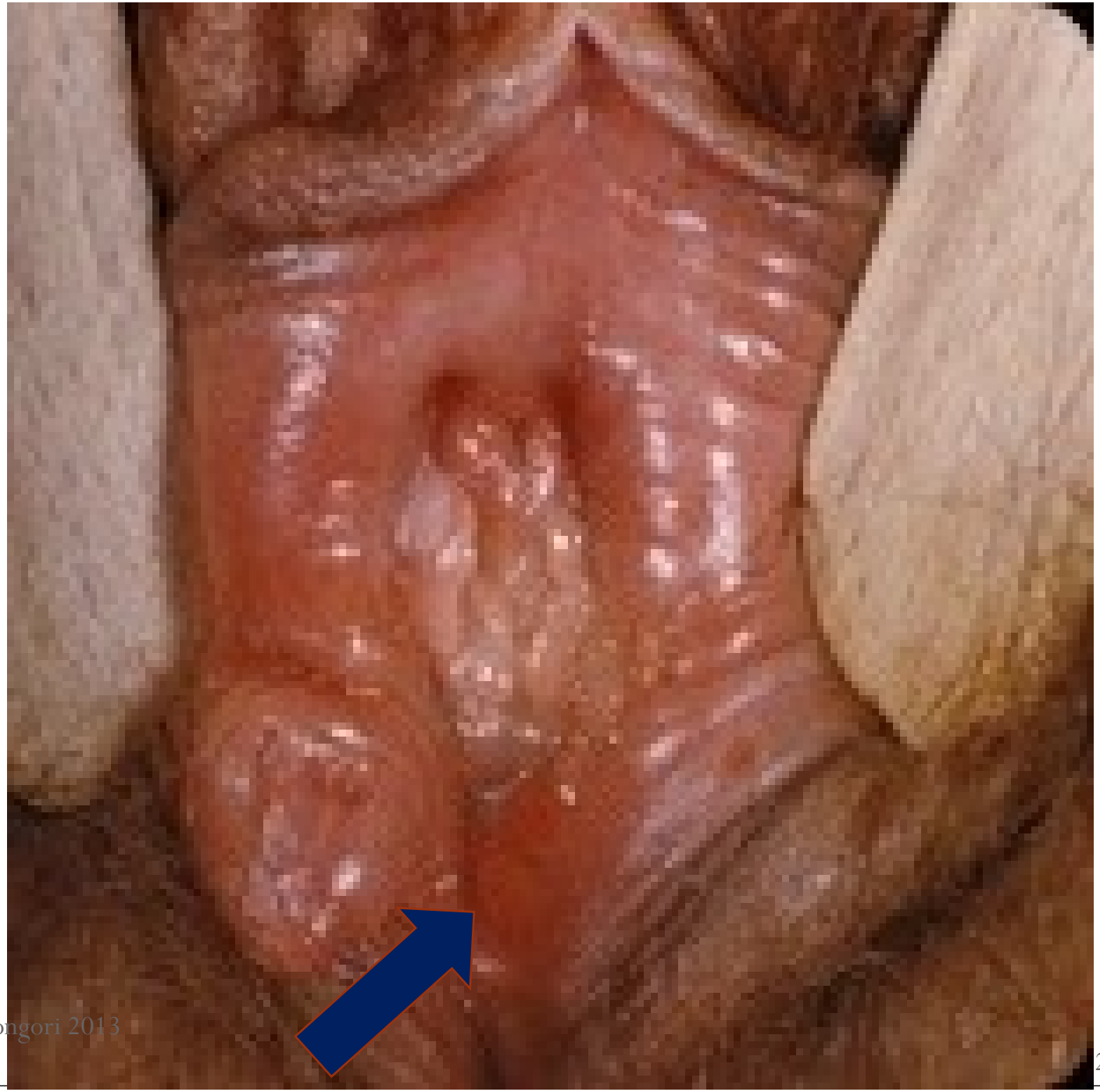
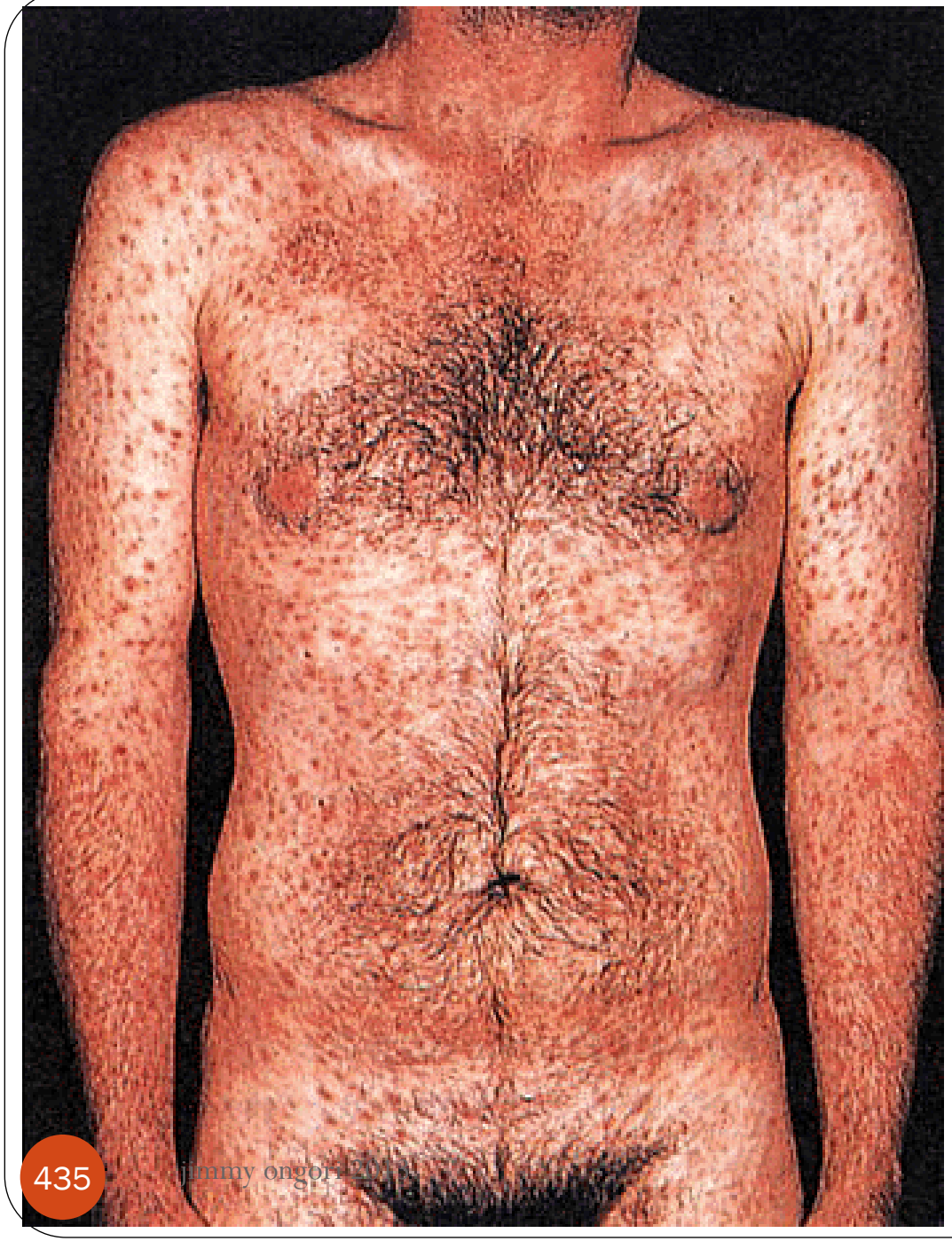


Secondary Syphilis

- Secondary disease 2-10 weeks after primary lesion
- Widely disseminated mucocutaneous rash
- Secondary lesions of the skin and mucus membranes are highly contagious
- Generalized immunological response



Generalized Mucocutaneous Rash of Secondary Syphilis

Latent Stage Syphilis

- Following secondary disease, host enters latent period
  - First 4 years = early latent
  - Subsequent period = late latent
- About 40% of late latent patients progress to late tertiary syphilitic disease

Tertiary syphilis

- Characterized by localized granulomatous dermal lesions (gummas) in which few organisms are present
- Granulomas reflect containment by the immunologic reaction of the host to chronic infection
- Late neurosyphilis develops in about 1/6 untreated cases, usually more than 5 years after initial infection
  - Central nervous system and spinal cord involvement
  - Dementia, seizures, wasting, etc.
- Cardiovascular involvement appears 10-40 years after initial infection with resulting myocardial insufficiency and death

Congenital Syphilis

- A pregnant syphilitic woman can transmit *T pallidum* to the fetus through the placenta beginning in the 10th to 15th weeks of gestation.
- Some of the infected fetuses die, and miscarriages result; others are stillborn at term.
- Others are born live but develop the signs of congenital syphilis in childhood: interstitial keratitis, Hutchinson's teeth, saddlenose, periostitis, and a variety of central nervous system anomalies. Adequate treatment of the mother during pregnancy prevents congenital syphilis.

Diagnostic Laboratory Tests

- **Specimens -** tissue fluid from early surface lesions for demonstration of spirochetes; blood serology
- **Darkfield Examination -** examined under oil immersion with darkfield illumination for typical motile spirochetes.
- Treponemes disappear from lesions within a few hours after the beginning of antibiotic treatment.
- **Immunofluorescence -** for typical fluorescent spirochetes.
- **Serologic Tests for Syphilis (STS) -** These tests use either nontreponemal or treponemal antigens

- **Treponemal Antibody Tests**
- Fluorescent Treponemal Antibody (FTA-ABS) Test - It is the first to become positive in early syphilis, is routinely positive in secondary syphilis, and usually remains positive many years after effective treatment. The presence of IgM FTA in the blood of newborns is good evidence of in utero infection (congenital syphilis).
- *Treponema pallidum*-Particle Agglutination (TP-PA) Test
- The most widely used **nontreponemal** antibody tests for syphilis are the RPR and VDRL tests, which measure IgG and IgM

Prevention & Treatment of Syphilis

- Penicillin (benzathine pen.) remains drug of choice
  - WHO monitors treatment recommendations
  - 7-10 days continuously for early stage
  - At least 21 days continuously beyond the early stage
- Prevention with barrier methods (e.g., condoms)
- Prophylactic treatment of contacts identified through epidemiological tracing

LEPTOSPIRAE

- Are tightly coiled, thin, flexible spirochetes 5–15 m long, with very fine spirals 0.1–0.2 m wide; one end is often bent, forming a hook.
- Actively motile, best seen using a darkfield microscope.
- It does not stain

Culture

- Grow best under aerobic conditions at 28–30 °C in serum-containing semisolid media. After 1–2 weeks the leptospirae produce a diffuse zone of growth near the top of the tube and later a ring of growth at a level in the tube corresponding to the level of the optimal oxygen tension for the organisms. T

Leptospira interrogans

- Leptospires are coiled, thin, highly motile organisms with hooked ends and two periplasmic flagella that permit burrowing into tissue.
- They stain poorly but can be seen microscopically by dark-field examination
- Leptospires require special media and conditions for growth; it may take weeks for cultures to become positive.

Epidemiology

- Leptospirosis is an important zoonosis with a worldwide distribution
- Rodents, esp rats are the most important reservoir, other wild mammals , domestic and farm animals may also harbor it
- Occurs most commonly in the tropics because the climate as well as the sometimes-poor hygienic conditions favor the pathogen's survival and distribution.
- Transmission of leptospires to humans may follow direct contact with urine, blood, or tissue from an infected animal or exposure to a contaminated environment; human-to-human transmission is rare.
- Since leptospires can survive in water for many months, water is an important vehicle in their transmission.

Pathogenesis & Clinical Findings

- Infection results from leptospires entering the body through breaks in the skin and mucus membranes (mouth, nose, conjunctivae).
- After an incubation period of 1–2 weeks, there is a variable febrile onset during which spirochetes are present in the bloodstream.
- They establish themselves in the organs (particularly liver and kidneys), producing hemorrhage and necrosis of tissue and resulting in dysfunction eg jaundice.
- After initial improvement, the 2nd phase develops, It manifests itself often as "aseptic meningitis" with intense headache, stiff neck, and pleocytosis of the cerebrospinal fluid.
- Nephritis and hepatitis may also recur, and there may be skin, muscle, and eye lesions.

- Weil's syndrome, the most severe form of leptospirosis, is characterized by jaundice, renal dysfunction, and hemorrhagic diathesis; by pulmonary involvement in many cases; and by mortality rates of 5–15%

Diagnostic Laboratory Tests

- **Specimens** - blood, cerebrospinal fluid, or tissues Urine should be collected using great care to avoid contamination.
- **Microscopic Examination** - Darkfield examination or thick smears stained by the Giemsa technique
- **Culture -** Whole fresh blood or urine can be cultured in Fletcher's semisolid or other medium.
- **Animal Inoculation -** intraperitoneal inoculation of guinea pigs with fresh plasma or urine. Within a few days, spirochetes become demonstrable in the peritoneal cavity; on the death of the animal (8–14 days), hemorrhagic lesions with spirochetes are found in many organs.

Treatment

- Mild leptospirosis - oral doxycycline, ampicillin, or amoxicillin, moderate or severe disease should be IV penicillin

Prevention, & Control

- These is animal infection; human infection is only accidental, Rats, mice, wild rodents, dogs, swine, and cattle are the principal sources of human infection.
- Leptospirae remain viable in stagnant water for several weeks; drinking, swimming, bathing, or food contamination may lead to human infection.
- Control - preventing exposure to potentially contaminated water and rodent control. Doxycycline, 200 mg once weekly during heavy exposure, is effective prophylaxis.

The *Haemophilus* Species

- Gram-negative, pleomorphic bacteria that require enriched media, usually containing blood or its derivatives, for isolation.
- Obligate Parasites of Man and Animals

- *Haemophilus influenzae* type b is an important human pathogen;
- *Haemophilus ducreyi,* a sexually transmitted pathogen, causes chancroid

Haemophilus influenzae

- Found on the mucous membranes of the upper respiratory tract in humans. An important cause of meningitis in children and occasionally causes RTI children and adults.
- The organisms are short, coccoid bacilli, sometimes occurring in pairs or short chains.
- Have a capsule.

Culture

- On chocolate agar, flat, grayish-brown colonies are present after 24 hours of incubation.
- *H influenzae* does not grow on sheep blood agar except around colonies of staphylococci ("satellite phenomenon").

Antigenicity

- Encapsulated *H influenzae* contains **capsular polysaccharides** of one of six types (a–f). - The capsular antigen
- The somatic antigens of *H influenzae* consist of outer membrane proteins.

Pathogenesis

- The capsule is antiphagocytic , The capsular antigen of type b *H influenzae* is the major virulence factor.
- Type b *H influenzae* causes meningitis, pneumonia and empyema, epiglottitis, cellulitis, septic arthritis, and occasionally other forms of invasive infection.
- The blood of many persons over age 3–5 years is bactericidal for *H influenzae,* and clinical infections are less frequent in such individuals.

Clinical Findings

- Enters through the respiratory tract. There may be local extension with involvement of the sinuses or the middle ear. *H influenzae* type b and pneumococci are two of the most common etiologic agents of bacterial otitis media and acute sinusitis.
- Reach the bloodstream and carried to the meninges or, in the joints to produce septic arthritis.
- Prior to the use of the conjugate vaccine, *H influenzae* was the most common cause of bacterial meningitis in children age 5 months to 5 years in the United States.

- Occasionally, a fulminating obstructive laryngotracheitis with swollen, cherry-red epiglottis develops in infants
- Pneumonitis and epiglottitis due to *H influenzae* may follow upper respiratory tract infections in small children and old or debilitated people. Adults may have bronchitis or pneumonia due to *H influenzae.*

Diagnostic Laboratory Tests

- **Specimens -** nasopharyngeal swabs, pus, blood, and spinal fluid for smears and cultures.
- **Direct Identification -** Commercial kits are available for immunologic detection of *H influenzae* antigens in spinal fluid.
- **Culture**
- Specimens are grown on IsoVitaleX-enriched chocolate agar until typical colonies appear. *H influenzae* is differentiated from related gram-negative bacilli by its requirements for X (heme) and V (nicotinamide-adenine dinucleotide) factors and by its lack of hemolysis on blood agar.

Treatment

- The mortality rate of untreated *H influenzae* meningitis may be up to 90%.
- Many strains of *H influenzae* type b are susceptible to ampicillin, but up to 25% produce -lactamase under control of a transmissible plasmid and are resistant.
- Essentially all strains are susceptible to the third-generation cephalosporins eg IV Cefotaxime

Epidemiology, Prevention, & Control

- Encapsulated *H influenzae* type b is transmitted from person to person by the respiratory route.
- Disease can be prevented by administration of **Haemophilus b conjugate vaccine** to children. Given in Kenya as the Hib component of Pentavalent vaccine at 6, 10 and 14 weeks of life
- Contact with patients suffering from *H influenzae* clinical infection poses little risk for adults but presents a definite risk for nonimmune siblings and other nonimmune children under age 4 years who are close contacts.

BRUCELLAE

- Small, Gram -VE cocobacilli, non-motile, non-spore forming.
- Brucellae grow aerobically.
- Some spp. Require supplemental carbon dioxide for primary isolation.
- Any peptone-based media enriched with blood or serum serve for in vitro cultivation.
- Isolation form clinical specimens require prolonged (≥ 30 days) incubation.
- Brucella strains always catalse-positive; but oxidase and urease and H_2S production vary.

- Genus Brucella divided into six spp. on basis of preferred hosts and cultural, metabolic and antigenic characteristics.

Epidemiology

- Brucellosis – zoonosis – all infections, derive directly or indirectly from animal exposure.
- Disease exists world-wide
- ***B. abortus*** found mainly in cattle, but others spp. like buffalo, camels
- ***B. Melitensis*** primary affects goats and sheep. Camels can be important source in some countries.

- ***B. Suis*** in domestic and swine, cause abattoir-assoc. human disease.
- ***B. Canis***: Least common cause of human disease.
- Animals: Brucellosis, Chronic Infection, persisting for life.
- Brucellae localization in reproductive organs, accounts for major manifestations – abortion and sterility.
- Brucellae shed in large numbers in: Milk, urine, products of infected animals.

- Brucellosis constitutes occupational risk for: farmers, veterinarians, abattoirs and Laboratory personnel.
- Routes of transmission to human include:
  - Direct contact with animals or their secretions, through cuts and skin abrasions.
  - Infected aerosols inhaled or inoculated into eye conjunctival sac.
  - Ingestion of unpasteurized dairy products.

- Meat products: rare source of infection because meat is rarely eaten raw and organisms are present in low number of muscle tissue.
- Blood and bone marrow may transmit disease when ingested in some cultures.
- Human-to-human transmission: Unusual, but rare cases suspected to be sexually transmitted.

Pathogenesis

- B. melitensis and B. suis, more virulent than B. abortus and B. canis.
- Infection with any B. species, can cause serious human disease.
- Once brucellae gain entry to body: PMN – Leukocytes attracted to inoculation site by chemotaxis.
- Normal human serum has limited bactericidal activity against brucellae, but it effectively opsonizes bacteria for phagocytosis by PMN – Leukocytes.
- Are Facultative intracellular, slowly dividing pathogens with capacity to survive and multiply within host phagocytic cells.

- Hematogenous dissemination then followed by localization of bacteria within organs rich in reticuloendothelial system, e.g. liver, spleen and B. marrow.

Clinical Manifestations

- Fever, sweats, malaise, anorexia, headache, backpain.
- Onset: acute or insidious, beginning within 2 to 4 weeks after inoculation.
- Malodorous sweat and peculiar mouth taste.
- Depression common.
- In comparison to plethora of somatic complaints, physical abnormalities are few.
- Mild lymphadenopathy reported in 10 to 20% of cases.
- Splenomegaly or hepatomegaly in 20 to 30% of cases.

Diagnostic Laboratory Tests

Culture - Brucella agar was specifically designed to culture *Brucella* species - Bone marrow and blood are the specimens from which brucellae are most often isolated.

- Most Labs. Now use rapid isolation techniques, e.g. BACTEC

- **Agglutination Test -** IgG agglutinin titers above 1:80 indicate active infection.
- Rose Bengal test

Treatment

- Brucellae may be susceptible to tetracyclines or ampicillin. Symptomatic relief may occur within a few days after treatment with these drugs is begun. However, because of their intracellular location, the organisms are not readily eradicated completely from the host. For best results, treatment must be prolonged. Combined treatment with a tetracycline (such as doxycycline) and either streptomycin for 2–3 weeks or rifampin for 6 weeks is recommended.

Prevention

- Prevention of human brucellosis depend on:

Control and elimination of Brucellosis in domestic animals.

- Effective attenuated live bacterial vaccines exist for:
  - B. abortus
  - B. melitensis
  - No vaccines for B. suis and B. canis
- No licensed human vaccine

Bordetella Pertussis

- Gram –VE Coccobaccili (rod-shaped)
- Obligate aerobe
- Optimum growth 35^0C-37^0 C (mesophile)
- Colonizes the respiratory tract
  - Whooping Cough (Pertussis)
- Specific to human hosts
- Approximately the size of 0.8 µm by 0.4 µm.
- pH - Found to grow better on media with a slightly acidic reaction

Virulence Factors

- **Adhesions -** Filamentous hemagglutinin (FHA), Pertactin, Fimbriae
- **Toxins**
  - **Pertussis Toxin (**PTX**) -** Colonizing factor
  - **Adenylate Cyclase Toxin (**CYA**) -** Invasive toxin
  - **Tracheal Cytotoxin (**TCT**)**

Clinical Findings

- Incubation period about 2 weeks, the "catarrhal stage" develops, with mild coughing and sneezing. During this stage, large numbers of organisms are sprayed in droplets, and the patient is highly infectious
- "paroxysmal" stage - the cough develops its characteristic "whoop" upon inhalation. This leads to rapid exhaustion and may be associated with vomiting, cyanosis, and convulsions. The "whoop" and major complications occur predominantly in infants; paroxysmal coughing predominates in older children and adults.

Diagnostic Laboratory Tests

- **Specimens** - A saline nasal wash is the preferred specimen.
- Direct Fluorescent Antibody (FA) Test
- **Culture -** cultured on solid medium agar.
- PCR is the most sensitive method

Treatment

- Erythromycin during the catarrhal stage of disease promotes elimination of the organisms and may have prophylactic value.
- Treatment after onset of the paroxysmal phase rarely alters the clinical course.
- Oxygen inhalation and sedation may prevent anoxic damage to the brain.

Prevention

- Every infant should receive three injections of pertussis vaccine during the first year of life
- In Kenya Pertussis vaccine is usually administered in combination with toxoids of diphtheria and tetanus and Hepatitis B and Haemophilus influenza type B as Pentavalent vaccine at 6, 10 and 14 weeks.
- Prophylactic administration of erythromycin for 5 days may also benefit unimmunized infants or heavily exposed adults.

CHLAMYDIA

- Chlamydiae that infect humans are divided into three *species—Chlamydia trachomatis, Chlamydophila (Chlamydia) pneumoniae,* and *Chlamydophila (Chlamydia) psittaci*
- In chlamydiae, the outer **cell wall** resembles the cell wall of gram-negative bacteria. It has a relatively high lipid content. It is rigid but does not contain a typical bacterial peptidoglycan.
- Lysozyme has no effect on chlamydial cell walls.

- Obligatory intracellular bacteria
- Infect columnar epithelial cells
- Survive by replication that results in the death of the cell
- Takes on two forms in its life cycle:
  - Elementary body (EB)
  - Reticulate body (RB)

Chlamydiaceae Family (species that cause disease in humans)

Species (genus)	Disease
C. trachomatis 2 biovars, non-LGV LGV	Trachoma, NGU, MPC, PID, conjunctivitis, Infant pneumonia, LGV
C. pneumoniae	Pharyngitis, bronchitis, pneumonia
C. psittaci	Psittacosis

Staining Properties

- Chlamydiae have distinctive staining properties. Elementary bodies stain purple with Giemsa stain, the larger, noninfective reticulate bodies stain blue with Giemsa stain.
- The Gram reaction of chlamydiae is negative or variable and is not useful in identification of the agents.

Characteristics of Host-Parasite Relationship

- The outstanding biologic feature of infection by chlamydiae is the balance that is often reached between host and parasite, resulting in prolonged persistence of infection. Subclinical infection is the rule—and overt disease the exception—in the natural hosts of these agents.
- Antibodies to several antigens of chlamydiae are produced by the infected host. These antibodies have little protective effect against reinfection. The infectious agent commonly persists in the presence of high antibody titers. Treatment with effective antimicrobial drugs for prolonged periods may eliminate the chlamydiae from the infected host.

Clinical Syndromes Caused by *C. trachomatis*

	Local Infection	Complication	Sequelae
Men	Conjunctivitis Urethritis Prostatitis	Reiter's syndrome Epididymitis	Chronic arthritis (rare) Infertility (rare)
Women	Conjunctivitis Urethritis Cervicitis Proctitis	Endometritis Salpingitis Perihepatitis Reiter's syndrome	Infertility Ectopic pregnancy Chronic pelvic pain Chronic arthritis (rare)
Infants	Conjunctivitis Pneumonitis Pharyngitis Rhinitis	Chronic lung disease?	Rare, if any

C. trachomatis Infection in Men

- Urethritis–One cause of non-gonococcal urethritis (NGU)
  - Majority (>50%) asymptomatic
  - Symptoms/signs if present: mucoid or clear urethral discharge, dysuria
  - Incubation period unknown

  Complicates to;
  - Epididymitis
  - Reiter's Syndrome - Rarely occurs in women

C. trachomatis Infections in Women

- Cervicitis - Majority (70%-80%) are asymptomatic
  - Local signs of infection, when present, include:
    - Mucopurulent endocervical discharge
- Urethritis - Usually asymptomatic
- Complications - Pelvic Inflammatory Disease (PID) (Salpingitis, Endometritis)
- Reiter's Syndrome

C. trachomatis Infections in Infants

- Perinatal clinical manifestations:
  - Inclusion conjunctivitis
  - Pneumonia

Diagnosis

- Culture
- Non-culture tests
  - Nucleic Acid Amplification Tests (NAATs)
  - Non-Nucleic Acid Amplification Tests (Non-NAATs)
  - Serology

Lymphogranuloma Venereum

- LGV is a sexually transmitted disease caused by *C trachomatis* and characterized by suppurative inguinal adenitis
- **Clinical Findings -** Several days to several weeks after exposure, a small, evanescent papule or vesicle develops on any part of the external genitalia, anus, rectum, or elsewhere. The lesion may ulcerate, but usually it remains unnoticed and heals in a few days. Soon thereafter, the regional lymph nodes enlarge and tend to become matted and painful.
- The nodes suppurate and discharge pus. In females and in homosexual males, the perirectal nodes are involved, with proctitis and a bloody mucopurulent anal discharge.
- chronic inflammatory process progresses to fibrosis, lymphatic obstruction, and rectal strictures. The lymphatic obstruction may lead to elephantiasis of the penis, scrotum, or vulva.

VIRUSES

DNA VIRUSES

Parvoviridae		
Hepadnaviridae		
Papillomaviridae		
Adenoviridae		
Herpesviridae		
poxviridae		

RNA VIRUSES

PICORNAVIRIDAE		
CALICIVIRIDAE		
Adenoviridae		
Herpesviridae		
poxviridae		

Introduction

- Viruses are the smallest infectious agents and contain only one kind of nucleic acid (RNA or DNA) as their genome.
- The nucleic acid is encased in a protein shell, which may be surrounded by a lipid-containing membrane. The entire infectious unit is termed a **virion**.
- Viruses are inert in the extracellular environment; they replicate only in living cells, being parasites at the genetic level.
- The viral nucleic acid contains information necessary for programming the infected host cell to synthesize virus-specific macromolecules required for the production of viral progeny.

- During the replicative cycle, numerous copies of viral nucleic acid and coat proteins are produced. The coat proteins assemble together to form the capsid, which encases and stabilizes the viral nucleic acid against the extracellular environment and facilitates the attachment and penetration by the virus upon contact with new susceptible cells. The virus infection may have little or no effect on the host cell or may result in cell damage or death.
- The universe of viruses is rich in diversity. Viruses vary greatly in structure, genome organization and expression, and strategies of replication and transmission. The host range for a given virus may be broad or extremely limited. Viruses are known to infect unicellular organisms such as mycoplasmas, bacteria, and algae and all higher plants and animals.

Terms & Definitions in Virology

- **Capsid:** The protein shell, or coat, that encloses the nucleic acid genome.
- **Capsomeres:** Morphologic units seen in the electron microscope on the surface of icosahedral virus particles.
- **Defective virus:** A virus particle that is functionally deficient in some aspect of replication.
- **Envelope:** A lipid-containing membrane that surrounds some virus particles. It is acquired during viral maturation by a budding process through a cellular membrane.

- **Nucleocapsid:** The protein-nucleic acid complex representing the packaged form of the viral genome.
- **Structural units:** The basic protein building blocks of the coat. They are usually a collection of more than one nonidentical protein subunit.
- **Subunit:** A single folded viral polypeptide chain.
- **Virion:** The complete virus particle. In some instances (eg, papillomaviruses, picornaviruses), the virion is identical with the nucleocapsid. In more complex virions (herpesviruses, orthomyxoviruses), this includes the nucleocapsid plus a surrounding envelope. This structure, the virion, serves to transfer the viral nucleic acid from one cell to another.

Bacteriophage Structure

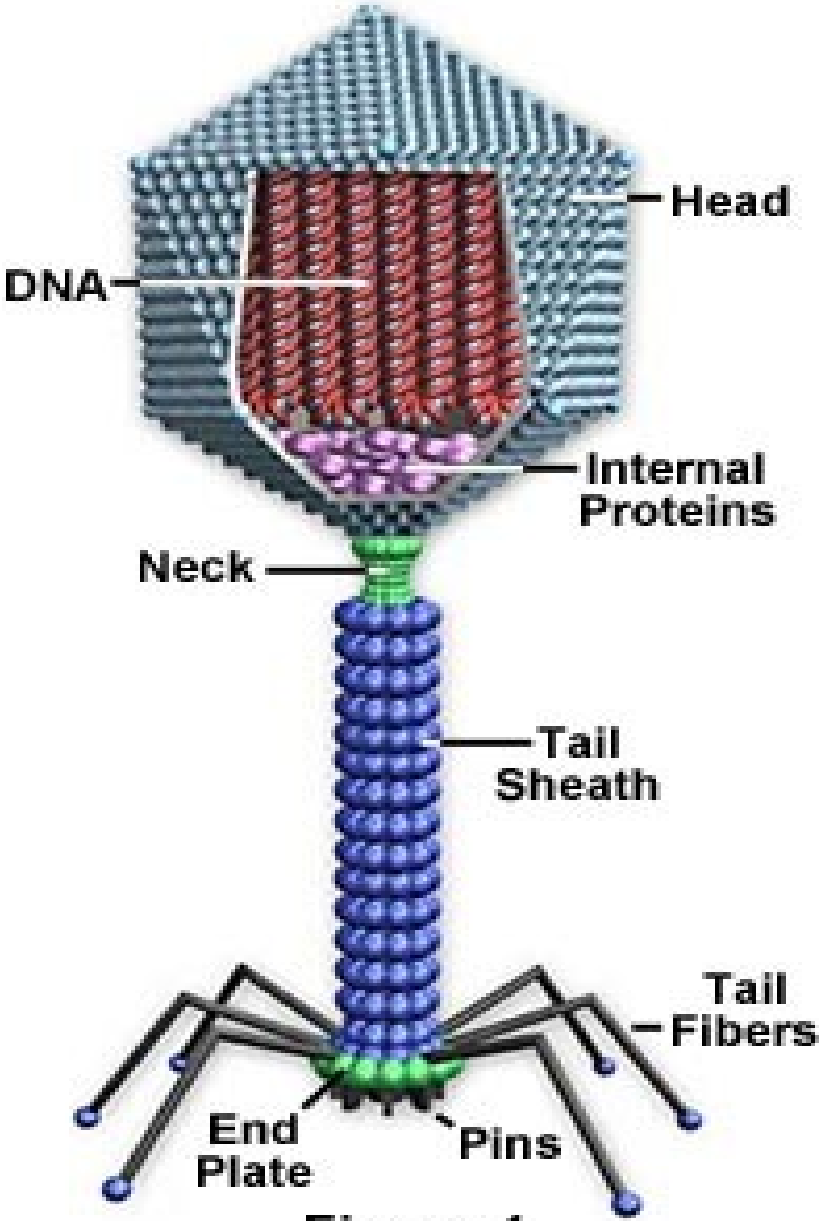
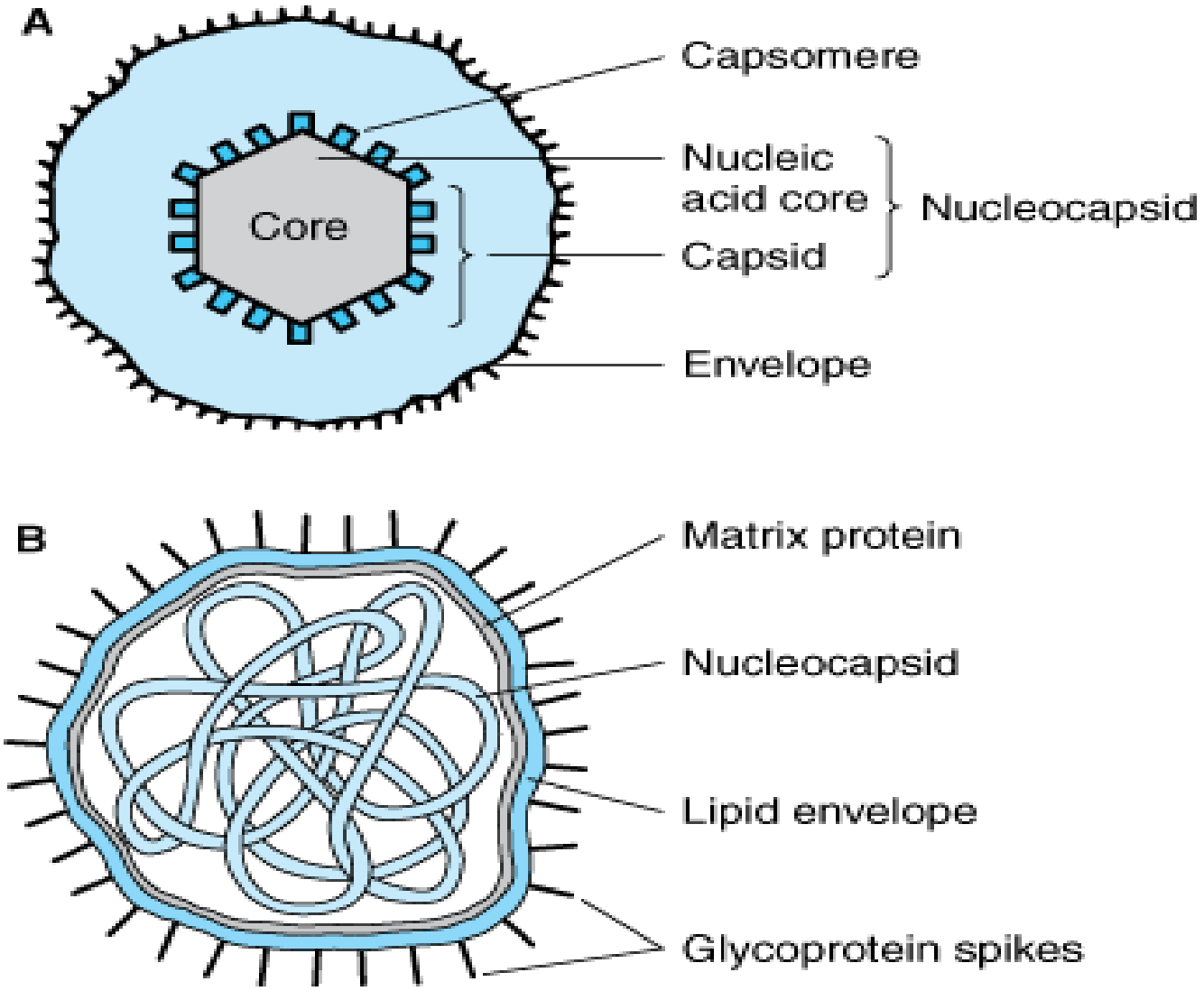


Figure 1

How are viruses named?

- Based on:
  - the disease they cause
    poliovirus, rabies virus
  - the type of disease
    murine leukemia virus
  - geographic locations
    Sendai virus, Coxsackie virus
  - their discovers
    Epstein-Barr virus
  - how they were originally thought to be contracted
    dengue virus ("evil spirit"), influenza virus (the "influence" of bad air)
  - combinations of the above
    Rous Sarcoma virus



Source: Brooks GF, Butel JS, Morse SA: *Jawetz, Melnick, & Adelberg's Medical Microbiology*, 24th Edition: http://www.accessmedicine.com

Viral structure

- Certain viruses contain ribonucleic acid (RNA), while other viruses have deoxyribonucleic acid (DNA). The nucleic acid portion of the viruses is known as the **genome.** The nucleic acid may be single-stranded or double-stranded; it may be linear or a closed loop; it may be continuous or occur in segments.
- The genome of the virus is surrounded by a protein coat known as a **capsid**, which is formed from a number of individual protein molecules called **capsomeres.** Capsomeres are arranged in a precise and highly repetitive pattern around the nucleic acid. A single type of capsomere or several chemically distinct types may make up the capsid. The combination of genome and capsid is called the viral **nucleocapsid.**

- A number of kinds of viruses contain **envelopes.** An envelope is a membrane like structure that encloses the nucleocapsid and is obtained from a host cell during the replication process. The envelope contains viral-specified proteins that make it unique. Enveloped viruses - herpes simplex, chickenpox, and infectious mononucleosis etc
- The nucleocapsids of viruses are constructed according to certain symmetrical patterns. The virus that causes tobacco mosaic disease, for example, has **helical symmetry.** In this case, the nucleocapsid is wound like a tightly coiled spiral. Other viruses take the shape of an icosahedron, and they are said to have **icosahedral symmetry.** In an icosahedron, the capsid is composed of 20 faces, each shaped as an equilateral triangle. Among the icosahedral viruses are those that cause yellow fever, polio, and head colds.

Bacteriophages;

- Are viruses that multiply within bacteria. These viruses are among the more complex viruses. They often have icosahedral heads and helical tails.
- Bacteriophages contain DNA and are important tools for viral research.

VIRAL REPLICATION

Viral life cycle consists of six stages within the host cell

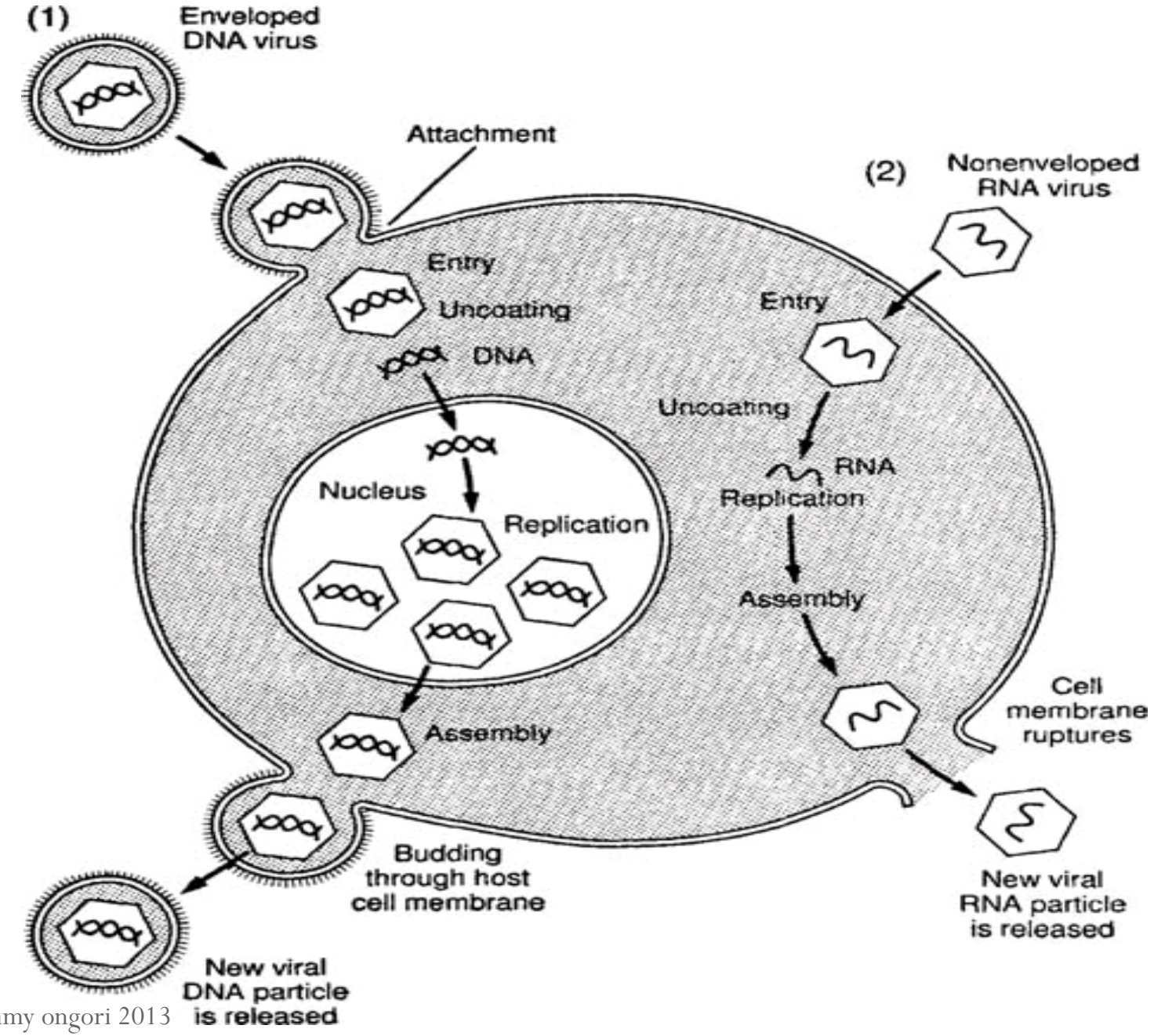
- Attachment
- Penetration
- Uncoating
- Multiplication
- Assembly
- Release

- During the process of **viral replication**, a virus induces a living host cell to synthesize the essential components for the synthesis of new viral particles. The particles are then assembled into the correct structure, and the newly formed virions escape from the cell to infect other cells.

1. The first step in the replication process is **attachment.** In this step, the virus adsorbs to a susceptible host cell. High specificity exists between virus and cell, and the envelope spikes may unite with cell surface receptors. Receptors may exist on bacterial pili or flagella or on the host cell membrane.

2. The next step is **penetration** of the virus or the viral genome into the cell. This step may occur by phagocytosis; or the envelope of the virus may blend with the cell membrane; or the virus may "inject" its genome into the host cell. The latter situation occurs with the bacteriophage when the tail of the phage unites with the bacterial cell wall and enzymes open a hole in the wall. The DNA of the phage penetrates through this hole.
3. The **replication** steps of the process occur next. The protein capsid is stripped away from the genome, and the genome is freed in the cell cytoplasm. If the genome consists of RNA, the genome acts as a messenger RNA molecule and provides the genetic codes for the synthesis of enzymes. The enzymes are used for the synthesis of viral genomes and capsomeres and the assembly of these components into new viruses.

- If the viral genome consists of DNA, it provides the genetic code for the synthesis of messenger RNA molecules, and the process proceeds.
- In some cases, such as in HIV infection, the RNA of the virus serves as a template for the synthesis of a DNA molecule. The enzyme reverse transcriptase catalyzes the DNA's production. The DNA molecule then remains as part of the host cell's chromosome for an unspecified period. From this location, it encodes messenger RNA molecules for the synthesis of enzymes and viral components.


(1)
Enveloped DNA virus
Attachment
(2)
Nonenveloped RNA virus
Entry
Uncoating
DNA
Nucleus
Replication
Assembly
Budding through host cell membrane
New viral DNA particle is released
Entry
Uncoating
RNA
Replication
Assembly
Cell membrane ruptures
New viral RNA particle is released

4. Once the viral genomes and capsomeres have been synthesized, they are assembled to form new virions. This **assembly** may take place in the cytoplasm or in the nucleus of the host cell. After the assembly is complete, the virions are ready to be released into the environment.

5. For the **release** of new viral particles, any of a number of processes may occur. For example, the host cell may be "biochemically exhausted," and it may disintegrate, thereby releasing the virions.

- For enveloped viruses, the nucleocapsids move toward the membrane of the host cell, where they force themselves through that membrane in a process called **budding.** During budding, a portion of cell membrane pinches off and surrounds the nucleocapsid as an envelope. The replication process in which the host cell experiences death is called the **lytic cycle** of reproduction.

- **Lysogeny.** Not all viruses multiply by the lytic cycle of reproduction. Certain viruses remain active within their host cells for a long period without replicating. This cycle is called the **lysogenic cycle.**
- An example of lysogeny occurs in HIV infection. In this case, the human immunodeficiency virus remains latent within the host T-lymphocyte. An individual whose infection is at this stage will not experience the symptoms of AIDS until a later date.

- Viruses infect all major groups of organisms: vertebrates, invertebrates, plants, fungi, bacteria but some viruses have a broader host range than others; however, none can cross the eukaryotic/prokaryotic boundary.

Factors that affect host range include:

- Whether the virus can get into the host cell; that is, does it have the correct attachment protein to bind to a receptor on the cell surface? For example, HIV is largely restricted to cells that have the CD4 antigen on their surface
- Whether the appropriate cellular machinery is available for the virus to replicate; for example, some DNA viruses can only replicate in dividing cells which have high enough levels of deoxyribonucleotides for viral DNA synthesis.
- If the virus can replicate, whether infectious virus can get out of the cell and spread the infection.

STRUCTURE

Viruses can have the following shapes;

1. Helical symmetry
2. Icosahedral symmetry

- An icosahedron is a solid with twenty faces . All faces of the icosahedron are identical.

3. Helical symmetry
4. Complex symmetry

FIVE BASIC STRUCTURAL FORMS OF VIRUSES IN NATURE

1. **Naked icosahedral** e.g. poliovirus, adenovirus, hepatitis A virus
2. **Naked helical** e.g. tobacco mosaic virus. So far no human viruses with this structure are known
3. **Enveloped icosahedral** e.g. herpes virus, yellow fever virus, rubella virus
4. **Enveloped helical** e.g. rabies virus, influenza virus, parainfluenza virus, mumps virus, measles virus
5. **Complex** e.g. poxvirus

- **UNCONVENTIONAL AGENTS**
- There are also the 'unconventional agents' sometimes known as 'unconventional viruses' or 'atypical viruses' - Up to now, the main kinds that have been studied are viroids and prions.

1. VIROIDS

- Viroids contain RNA only. They are small (less than 400 nucleotides), single stranded, circular RNAs. The RNAs are not packaged, do not appear to code for any proteins, and so far have only been shown to be associated with plant disease. However, there are some suggestions that somewhat similar agents may possibly be involved in some human diseases.

- **Hepatitis delta virus**
- At present, the only known human disease agent to resemble viroids is hepatitis delta virus (HDV).
- HDV has a very small RNA genome compared to most viruses, although it is somewhat larger than viroids.

2. PRIONS

- Prions contain protein only. They are small, proteinaceous particles and there is controversy as to whether they contain any nucleic acid, Examples of prion-caused human diseases are Kuru, Creutzfeldt-Jakob disease and Gerstmann-Straussler syndrome.
- Prions also cause scrapie in sheep.

LENTIVIRUSSES

Characteristics:

- Members are nononcogenic and may be cytocidal
- Infect cells of the immune system
- Proviruses remain permanently associated with cells
- Cause slowly progressive, chronic diseases
- Replication is usually species-specific
- Group includes the causative agents of AIDS

Disinfection & Inactivation

- HIV is completely inactivated by treatment for 10 minutes at room temperature with any of the following: 10% household bleach, 50% ethanol, 35% isopropanol, 1% Nonidet P40, 0.5% Lysol, 0.5% paraformaldehyde, or 0.3% hydrogen peroxide. The virus is also inactivated by extremes of pH (pH 1.0, pH 13.0).
- HIV is readily inactivated in liquids or 10% serum by heating at 56 °C for 10 minutes, but dried proteinaceous material affords marked protection.

HUMAN IMMUNODEFICIENCY VIRUS AND AIDS

- The world pandemic of AIDS has been with us for more than twenty five years and shows no signs of abatement. Three million people around the world die of AIDS each year and, so far, more than 25 million people have died of the disease. Today, at least 33 million people are infected and there are more than 14,000 new infections every day.
- AIDS is caused by Human Immunodeficiency Virus (HIV) which is found in all cases of the disease. The primary targets of HIV are activated CD4+ T4 helper lymphocytes but the virus can also infect several other cell types including macrophages. It is the loss of T4 helper lymphocytes that leads to immunosuppression in the patient and the consequent fatal opportunistic infections.

- HIV is a lentivirus, a class of retrovirus. The name lentivirus means slow virus, so called because these viruses take a long time to cause overt disease.
- Most lentiviruses target cells of the immune system and thus disease is often manifested as immunodeficiency.
- There are five known serogroups of lentivirus that infect primates, sheep and goats, horses, cats, and cattle.

- **Two types of HIV**: HIV-1 and HIV-2. These cause clinically indistinguishable disease, although the time to disease onset is longer for HIV-2.
- The worldwide epidemic of HIV and AIDS is caused by HIV-1 while HIV-2 is mostly restricted to west Africa.
- Lentiviruses integrate into the host cell genome as a provirus in the same manner as other retroviruses
- HIV can lie dormant in the proviral form within a cell for many years, especially in resting (memory) CD4+ T4 lymphocytes, and may set up a lifelong infection. When these cells become reactivated, viral production occurs again and ultimately destroys the cell.

- Although HIV may disappear from the cells of the circulation, replication and budding continue to occur in other tissues in the absence of chemotherapy.
- HIV can be detected by the presence of anti-HIV antibodies or by the presence of the virus itself using (PCR) that detects viral RNA.
- From the original infection, there is usually a period of 8 to 10 years before the clinical manifestations of AIDS occur; however, this period may be two years or less. Approximately 10% of patients succumb to AIDS within 2 to 3 years.
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Acute infection (acute retroviral syndrome)

- This period lasts for 6 to 12 weeks after initial infection until anti-HIV antibodies are detectable.
- If acquired by sexual activity, the virus enters the body in infected macrophages in semen or vaginal secretions. Dendritic cells in the mucosal linings bind the virus shed by macrophages and carry it to the lymph nodes where $CD4^+$ T4 cells become infected. During the course of the disease, the virus migrates to other cell types.
- Initially, HIV infection produces a mild disease that is self-limiting. This is not seen in all patients and about 30% remain asymptomatic during the initial period of infection. In the period immediately after infection, virus titer rises (about 4 to 11 days after infection) and continues at a high level over a period of a few weeks.

- Patient experiences some symptoms (fever, rash, swollen lymph glands) but none of these is life-threatening. There is an initial fall in the number of $CD4^+$ cells and a rise in $CD8^+$ cells but they quickly return to near normal. At this stage virus titers are very high with as many as one hundred million virus particles per milliliter of plasma.
- There is a "**window period**" of seronegativity during which an infected person does not give a positive western blot HIV test or ELISA, even though the viral load is high and the patient may exhibit some symptoms.
- This seronegative period can last for six months before seroconversion although the latter usually occurs between one and four weeks after infection.

A strong cell-mediated and humoral anti-HIV immune defense

- Cytotoxic B and T lymphocytes mount a strong defense and virus largely disappears from the circulation. After the increased cell-mediated immune response, there is a rise in humoral antibodies. During this period of strong immune response to the virus, more than 10 billion new HIV particles are produced each day but they are rapidly cleared by the immune system and have a half life of only 5 to 6 hours.
- At this stage, most of this virus is coming from recently infected proliferating $CD4^+$ cells. Thus, the virus is destroying the very cells that are proliferating to protect against it. The infected cells that are producing this virus are destroyed either by the immune system or by the virus and have a half life about 1 day. However, the rate of production of $CD4^+$ cells can compensate for the loss of cells and a steady state is set up in which most CD4+ cells are uninfected.

- Although activated, proliferating CD4+ cells are destroyed by the immune system, a small fraction of the infected cells survive long enough to revert back to the resting memory state (as do non-infected $CD4^+$ memory cells). The resting memory cells (also referred to as anamnestic T cells) do not express viral antigens but do carry a copy of the HIV genome which remains latent until the cells are reactivated by antigen. These memory cells may survive many years and constitute a reservoir that is very important in drug-based therapy.
- During this period, the virus disseminates to other regions including to lymphoid and nervous tissue. This is the most infectious phase of the disease.

A latent reservoir

- As a result of the strong immune defence, the number of viral particles in the blood stream declines and the patient enters *clinical latency*.
- Little virus can now be found in the bloodstream or in peripheral blood lymphocytes and, initially, the number of blood CD4+ cells is only slightly decreased.
- Nevertheless, the virus persists elsewhere, particularly in lymph nodes and here viral replication continues
- Although the number of HIV particles in the bloodstream is much reduced during clinical latency, the virus is detectable.

- After the initial peak of virus, the virus reaches a "set point" during latency. This set point predicts the time of onset of clinical disease.
- With less than 1000 copies/ml of blood, disease will probably occur with a latency period of more than 10 years. With less than 200 copies/ml, disease does not appear to occur at all.
- Most patients with more than 100,000 copies per ml, lose their CD4+ cells more rapidly and progress to AIDS before 10 years.
- Most untreated patients have between 10,000 and 100,000 copies per ml in the clinical latency phase.

Loss of $CD4^+$ cells and collapse of the immune response

- One reason that the immune system fails to control HIV infection is that the $CD4^+$ T helper cells are the target of the virus. Also follicular dendritic cells can be infected with HIV and these also diminish in number over time.
- Moreover, dendritic cells present antigen to $CD4^+$ cells and may bring the virus into contact with these cells at the time that they are stimulated to proliferate by antigen.

During the course of infection, there is a profound loss of the specific immune response to HIV because:

1. responding CD4+ cells become infected. The cells that proliferate to respond to the virus are infected and killed by it

2. epitope variation can lead to escape of HIV from the immune response
3. activated CD4+ T cells are susceptible to **apoptosis**. Spontaneous apoptosis of uninfected $CD4^+$ and $CD8^+$ T cells occurs in HIV-infected patients.
4. the number of follicular dendritic cells falls over time, resulting in diminished capacity to stimulate CD4+ cells

- There is thus a decline of $CD4^+$ cells with especially a loss of those specific to HIV. This occurs from the very beginning of infection and is permanent (unless chemotherapy intervenes). Near the end stage of AIDS, $CD8^+$ cells also decline precipitously.
- During the course of HIV infection, most CD4+ cells are never actually infected by the virus but die from some other means.

Onset of disease - AIDS

- The period of clinical latency varies in length from as little as 1 to 2 years to more than 15 years. Onset of AIDS is rare in less than 3 years except in children.
- Eventually, the virus can no longer be controlled as helper CD4+ (T4) cells are destroyed. Ironically, the killer cells needed to control HIV also damage the helper T cells that they need to function efficiently.
- With the lack of $CD4^+$ cells, new cytotoxic T cell responses cannot occur as helper cells are lacking and such new responses are required as the virus mutates. As the CD4+ cells fall below 200 per cu mm, virus titers rise rapidly and immune activity drops precipitously.

- It is the loss of immune competence that enables normally benign opportunistic parasites such as viruses, fungi or protozoa to cause infections
- Once AIDS develops, patients rarely survive more than two years without chemotherapeutic intervention.
- There is considerable variability at this stage. Some patients with clinical AIDS do survive for several years while others who appear relatively healthy can suddenly succumb to a major opportunistic infection.
- It is the onset of HIV-associated cancers and opportunistic infections that defines AIDS proper. At this stage, also, syncytium-inducing HIV appear in many (about half) AIDS patients. These are more CD4+ cell tropic than the initially infecting HIV and this contributes to the rapid loss of CD4+ cells in later stages of the disease.

HERPES VIRUSES

INTRODUCTION

- Herpes viruses are a leading cause of human viral disease, second only to influenza and cold viruses. They are capable of causing overt disease or remaining silent for many years only to be reactivated, for example as shingles.
- The name herpes comes from the Latin *herpes* which, in turn, comes from the Greek word *herpein* which means to creep. This reflects the creeping or spreading nature of the skin lesions caused by many herpes virus types.
- There are at least 25 viruses in the family Herpesviridae.
- Eight or more herpes virus types are known to infect man frequently.

HERPES VIRUS TYPES THAT INFECT HUMANS

- Herpes simplex virus Type 1 (HSV-1)
- Herpes simplex virus Type 2 (HSV-2)
- Epstein Barr virus (EBV)
- Cytomegalovirus (CMV)
- Varicella Zoster Virus (VZV)
- Human herpes virus 6 (exanthum subitum or roseola infantum)
- Human herpes virus 8 (Kaposi's sarcoma-associate herpes virus)

- Once a patient has become infected by herpes virus, the infection remains for life. The initial infection may be followed by latency with subsequent reactivation.
- Herpes viruses infect most of the human population and persons living past middle age usually have antibodies to most of the above herpes viruses with the exception of HHV-8.

HERPES SIMPLEX VIRUS (HSV)

- These are very large viruses.
- There are two types, HSV-1 and HSV-2 with very similar characteristics

Pathogenesis

- The hallmark of herpes infection is the ability to infect epithelial mucosal cells or lymphocytes. The virus then travels up peripheral nerves to a nucleated neurone where it may stay for years followed by reactivation.
- A reddened area gives rise to a macula which crusts to form a papula. The fluid in this blister is full of virus. As long as the virus is kept moist it can remain infectious

- Herpes simplex 1 and 2 can infect both humans and other animals but only humans show symptoms of disease.
- HSV-1 and HSV-2 first infect cells of the mucoepithelia or enter through wounds. They then frequently set up latent infections in neuronal cells.
- Both types of HSV can also persistently infect macrophages and lymphocytes.
- Once epithelial cells are infected, there is replication of the virus around the lesion and entry into the innervating neurone. The virus travels along the neurone (by a process called retrograde transport) to the ganglion.
- In the case of herpes infections of the oral mucosa, the virus goes to the trigeminal ganglia whereas infections of the genital mucosa lead the virus entering the sacral ganglia.
- Vesicles containing infectious virus are formed on the mucosa and the virus spreads. The vesicle heals and there is usually no scar as a result.

Epidemiology

- HSV 1 and 2 infections are life-long and although latency is soon set up, the infected patient can infect others as a result of recurrence.
- The virus is found in the lesions on the skin but can also be present in a variety of body fluids including saliva and vaginal secretions.
- HSV-1 is usually spread mouth to mouth (kissing or the use of utensils contaminated with saliva) or by transfer of infectious virus to the hands after which the virus may enter the body via any wound or through the eyes. A large proportion of the population has evidence of HSV-1 infection as judged by antibodies.
- As a result of poor hygiene in underdeveloped countries, HSV-1 antibodies are found in more than 90% of children.
- HSV-2 is normally spread sexually and is found in the anus, rectum and upper alimentary tract as well as the genital area.

- In addition an infant can be infected at birth by a genitally-infected mother. The infant can also be infected *in utero* if the mother's infection spreads.
- Anyone who comes in contact with fluid containing infectious virus is at risk.
- As might be expected, HSV-2 infections are more prevalent later in life as the number of sexual contacts increases. Thus, the lowest rates of infection are found in children and the highest rates in prostitutes among whom as many as 80% are infected with HSV-2.

Diseases caused by Herpes Simplex Viruses

- Herpes simplex 1 and 2 are frequently benign but can also cause severe disease. In each case, the initial lesion looks the same. A clear vesicle containing infectious virus with a base of red (erythomatous) lesion at the base of the vesicle. From this pus-containing (pustular), encrusted lesions and ulcers may develop.

1. Oral herpes - Cold sores - Can be the result of an HSV-1 or an HSV-

2 infection. Because of the association of HSV-2 with sexual transmission,

infections in children are usually the result of HSV-1

2. Herpes keratitis - This is an infection of the eye and is primarily caused by HSV-1. It can be recurrent and may lead to blindness.

3.Herpes whitlow - disease of persons who come in contact with herpes-infected body secretions can be caused by either type of HSV and enters the body via small wounds on the hands. It can also be caused by transfer of HSV-2 from genitals to the hands.

4. Herpes gladiatorum

5. Eczema herpeticum

6. Genital herpes - Genital herpes is usually the result of HSV-2 with about 10% of cases being the result of HSV-1. Primary infection is often asymptomatic but many painful lesions can develop on the glans or shaft of the penis in men and on the vulva, vagina, cervix and perianal region of women.

- Secondary episodes of genital herpes, which occur as a result of reactivation of virus in the sacral ganglion, are frequently less severe (and last a shorter time) than the first episode.
- Whether there is an apparent active disease or not, an infected patient remains infectious without overt symptoms. Clearly, these persons are very important in the spread of herpes infection.

7. HSV proctitis - This is an inflammation of the rectum and the anus

8. HSV Encephalitis - This is usually the result of an HSV-1 infection and is the most common sporadic viral encephalitis.

9. HSV Meningitis - This is the result of an HSV-2 infection.

10.HSV infection of neonates

This results from HSV-2 and is often fatal, although such infections are rare. Infection is especially possible if the mother is shedding virus at the time of delivery.

Diagnosis of HSV Infections

- Cells may be obtained from the base of the lesion (called a Tzank smear) and histochemistry performed.
- The cells can also be stained with specific antibodies in an immunofluorescence test
- It is also possible to detect viral DNA by *in situ* hybridization
- Virus can be isolated from biopsy specimens, that is from the lesions, and grown on tissue culture cells

HSV chemotherapy

- Acycloguanosine (acyclovir), famciclovir and valacyclovir. It should be noted that these drugs act against the replicating virus and therefore they are ineffective against latent virus.
- Since once the virus infects, the patient has it for life, the best option is to avoid infection by not coming in contact with the virus.
- Patients with genital herpes should avoid intercourse when they have the prodromal itching symptoms or an active lesion.

VARICELLA-ZOSTER VIRUS (ALSO KNOWN AS HERPES ZOSTER VIRUS, HUMAN HERPES VIRUS-3)

- Zoster means girdle from the characteristic rash that forms a belt around the thorax in many patients. The structure of Varicella virus is very similar to Herpes Simplex virus
- **Diseases caused by Varicella-Zoster virus**
- This virus causes two major diseases, chicken-pox (Varicella), usually in childhood, and shingles, later in life. Shingles (Zoster) is a reactivation of an earlier varicella infection.

Chicken Pox

- This virus is highly infectious and even if we do not remember getting it, more than 90% of the population antibodies against varicella proteins. In the household of an infected patient, 90% of contacts who have hitherto not had the disease will get it (unless vaccinated). It is spread by respiratory aerosols or direct contact with skin lesions.

Shingles

- After the infectious period, the virus may migrate to the ganglia
- The virus may then be reactivated under stress or with immune suppression. This usually occurs later in life.
- The skin lesions are different from those in chicken pox, being restricted to small areas of the skin, usually in the thorax
- Patients with AIDS often exhibit multi-dermatomal recurrence of varicella infection. There is also a chronic verricous form in some AIDS patients.

EPSTEIN- BARR VIRUS

- Epstein-Barr virus is the causative agent of Burkitt's lymphoma in Africa, nasal pharyngeal carcinoma in the orient and infectious mononucleosis in the west.

Burkitt's lymphoma

- The association between EBV and Burkitt's lymphoma has long been established. This is a tumor of the jaw and face found in children. The tumor cells show evidence of EBV DNA and tumor antigens and patients show a much higher level of anti-EBV antibodies than other members of the population.
- This lymphoma is endemic in equatorial Africa . Why this is so is unclear but there is probably a genetic reason possibly involving an association with malaria. Persons who are resistant to malaria appear to be susceptible to progression to the lymphoma.

- **Nasopharyngeal cancer** - This disease is also associated with EBV. There may be a genetic predisposition to the development of EBV cancers
- **Oral hairy leukoplakia -** This EBV-associated disease results in lesions in the mouth and has increased in frequency recently as it is an opportunistic infection of HIV-infected patients
- **Infectious mononucleosis**

CYTOMEGALOVIRUS

Cytomegalovirus has the largest genome of all herpes viruses and appears only to replicate in human cells. Its name

derives form the fact that, like other herpes viruses, it can form multinucleated cells (syncytia) with characteristically

staining inclusions.

Transmission

- Cytomegalovirus infection is found in significant proportion of the population. As with Epstein-Barr virus (also spread in saliva), seropositivity increases with age.
- The virus is spread in most secretions, particularly saliva, urine, vaginal secretions and semen
- Cytomegalovirus infection is therefore sexually transmitted. It can also spread to a fetus in a pregnant woman and to the newborn via lactation,
- In the hospital, the virus can also be spread via blood transfusions and transplants.

Pathogenesis

- Cytomegalovirus causes no symptoms in children and at most mild disease in adults

Congenital disease

- During a primary infection of the mother, the virus can spread via the placenta to the fetus and congenital abnormalities can occur; in fact, this virus is the most common viral cause of congenital disease.

Disease in immunosuppressed patients

- In patients who have received an organ transplant or have an immunosuppressive disease (e.g. AIDS), cytomegalovirus can be a major problem. Particularly important is cytomegalovirus-retinitis in the eye which occurs in up to 15% of all AIDS patients. In addition, interstitial pneumonia, colitis, esophagitis and encephalitis are seen in some patients.

HEPATITIS VIRUSES

- Several diseases of the liver, collectively known as hepatitis, are caused by viruses. The viruses involved, five of which have been reasonably well characterized, come from a wide range of virus families.
- Hepatitis A virus is a picornavirus, a small single strand RNA virus;
- Hepatitis B virus belongs to the hepadnavirus family of double stranded DNA viruses;
- Hepatitis C virus is a flavivirus, a single stand RNA virus;
- Hepatitis E, also an RNA virus, is similar to a calicivirus.
- Hepatitis D which is also known as Delta agent is a circular RNA that is more similar to a plant a viroid than a complete virus.

HEPATITIS A VIRUS

- This picornavirus is the causative agent of infectious hepatitis

HEPATITIS B VIRUS

- Human hepatitis B virus belongs to the hepadnavirus family and causes serum hepatitis.
- It infects humans and chimpanzees
- HBV is a DNA virus and is enveloped. The DNA is only partly double stranded
- It is heat- and pH-resistant.

- Depending on the patient's immune response, infection by HBV can be asymptomatic, chronic or acute.
- Humans are the only reservoir for HBV. The virus is spread via contact with body fluids – blood, semen and various secretions eg vaginal fluids, menstrual blood, saliva and milk.
- Although injection of blood is the most common route of infection, the virus can also be contracted via sexual intercourse (particularly male to male) and perinatally.
- About 5% of people infected by HBV get a chronic infection. Up to one quarter of these chronically-infected patients will die of some form of liver disease.

Pathology

- HBV enters the body in the bloodstream and targets hepatocytes
- The incubation period is 60-90 days
- The first sign of infection is the characteristic appearance of HBsAg in infected cells.
- The symptoms are immune-mediated, resulting from inflammation and cell-mediated (cytotoxic T cell) responses to HBsAg on the surface of hepatocytes.
- Chronic HBV infection can lead to chronic hepatitis. This leads to cirrhosis of the liver in up to a quarter of patients within five years. Of these patients, up to one quarter will develop hepatocellular carcinoma or liver failure. Both of these are fatal in the absence of a liver transplant.

Diagnosis

- Serum hepatitis is usually first diagnosed from the clinical symptoms. Liver enzymes are also detected in the bloodstream during the symptomatic phase.
- An acute infection can be distinguished from a chronic infection by the presence of antibodies (IgM) against HBcAg. Tests that detect HBsAg and HBcAg
- The presence of HBeAg is the best marker for infectious virus.
- HBV can also be detected in the laboratory by immunohistochemistry (fi

Carcinogenesis

- It is clear that individuals who are HBsAg positive are at a much higher risk of hepatocellular carcinoma than those who are negative.
- In patients with chronic hepatitis, there is destruction of hepatocytes as a result of the immune response to the virus. This results in regeneration (by cell division) of liver cells that may ultimately cause the cancer.
- Although the virus does not integrate during the course of normal replication, parts of the HBV genome are found integrated into the DNA of hepatocellular carcinoma patients.
- Hepatocellular carcinoma takes many years to develop

HEPATITIS C VIRUS

- Hepatitis C is a flavivirus that causes non-A, non-B hepatitis

- **MEASLES (RUBEOLA)**

  The word measles is derived from the German word for blister.

- Before the advent of the current measles vaccine, there were about 500,000 cases of measles in the United States per year; almost everyone got the measles. But since 1963, the number has fallen precipitously (figure 1B) with a low of only 86 cases in 2001, all of which seem to be imported. In the less developed world, measles still takes its toll with an estimated 30 million illnesses and 770,000 measles-caused deaths in 2000 of which 58% were in Africa

- **PATHOGENESIS AND DISEASE** (figure 2)

  Infection is via an aerosol route and the virus is very contagious. It replicates initially in the upper/lower respiratory tract, followed by replication in lymphoid tissues leading to viremia and growth in a variety of epithelial sites. The disease develops 1 - 2 weeks after infection.

- Uncomplicated disease is characterized by the following:
- Fever of 101 degrees Fahrenheit (38.3 C) or above
- Respiratory tract symptoms: running nose (coryza) and cough
- Conjunctivitis (table 2)
- Koplik's spots on mucosal membranes (table 2) - small (1 - 3mm), irregular, bright red spots, with bluish-white speck at center. The patient may get an enormous number and red areas may become confluent (see below).
- Maculopapular rash which extends from face to the extremities. This seems to be associated with T-cells targeting infected endothelial cells in small blood vessels (table 2) (see below).

- The infection is prostrating but recovery is usually rapid. The peak of infectiousness is before the onset of obvious symptoms (Koplik's spots, rash). Note some virus shedding occurs during the disease phase, so spread of the virus to other individuals can be somewhat reduced by minimizing contact with others.
- The cell mediated response is important since patients with agamma-globulinemia recover normally. Measles tends to be more severe in adults and the very young (under 5 years of age) and is less severe in older children and teenagers.
- **Complications of measles**
- If a patient has an impaired cell-mediated immune response, there is continued growth of the virus in the lungs leading to giant cell pneumonia (such patients may not have a rash). This is rare, but often fatal. The reason for the giant cells is that, since F protein can function at physiological pH, it can facilitate cell-cell fusion.
- Since virus grows in epithelia of the nasopharynx, middle ear and lung, all of these sites may then be susceptible to secondary bacterial infection. Otitis media and bacterial pneumonia are quite common.
- The outcome of the disease is affected by the nourishment of the patient and access to medical care. Measles is still a major killer in underdeveloped countries and several studies in areas with severe vitamin A deficiency problems have found that vitamin A treatment of children with measles has resulted in reduction in morbidity and mortality. Pneumonia accounts for 60% of deaths from measles.
- One in 1000 cases may get encephalitis a few days after the rash disappears. Most patients (90%) survive encephalitis but there may be complications such as deafness, seizures and mental disorders.

HUMAN IMMUNODEFICIENCY VIRUS AND AIDS

Human Immunodeficiency Virus: Historical Background

1981	Doctors in US recognized PCP in homosexual males, a condition previously unreported in healthy adults • Later recognized that all these patients were immunosuppressed
1983/4	Scientist described the cause of this acquired immunodeficiency syndrome (AIDS) as a retrovirus – Lymphadenopathy Associated Virus (LAV) – AIDs Associated Retrovirus (ARV) – Human T- lymphotrophic Virus Ш (HTLV-Ш)
1984	First case described In Kenya
1986	Human Immunodeficiency Virus (HIV) accepted as international designation for the retrovirus in a WHO consultative meeting

Human Immunodeficiency Virus: Historical Background (cont.)

1996	ARVs available in the world
1997	ARVs available in Kenya private sector
2003	ARVs available in Kenya public sector
2005	54,000 patients on ART
2010	Approx.426,870 patients on ART

HIV VIRUS

- AIDS is caused by Human Immunodeficiency Virus (HIV), A retrovirus from the Lentivirus family.
- The primary targets of HIV are activated $CD4^+$ T4 helper lymphocytes but the virus can also infect several other cell types including macrophages.
- Loss of T4 helper lymphocytes leads to immunosuppression in the patient and the consequent fatal opportunistic infections.
- Genetic material consists of a single-stranded ribonucleic acid (RNA)

The Biology OfThe HIV

- HIV is a lentivirus, a class of retrovirus.
- The name lentivirus means slow virus, so called because these viruses take a long time to cause overt disease.
- Most lentiviruses target cells of the immune system and thus disease is often manifested as immunodeficiency.
- There are five known serogroups of lentivirus that infect primates, sheep and goats, horses, cats, and cattle.
- There are two types of HIV: HIV-1 and HIV-2. These cause clinically indistinguishable disease, although the time to disease onset is longer for HIV-2. The worldwide epidemic of HIV and AIDS is caused by HIV-1 while HIV-2 is mostly restricted to west Africa.

- **HIV – 1**
  - Is found worldwide
  - Is the main cause of the worldwide pandemic
- **HIV – 2**
  - Is mainly found in West Africa, Mozambique and Angola.
  - Causes a similar illness to HIV – 1
  - Less efficiently transmissible rarely causing vertical transmission
  - Less aggressive with slower disease progression

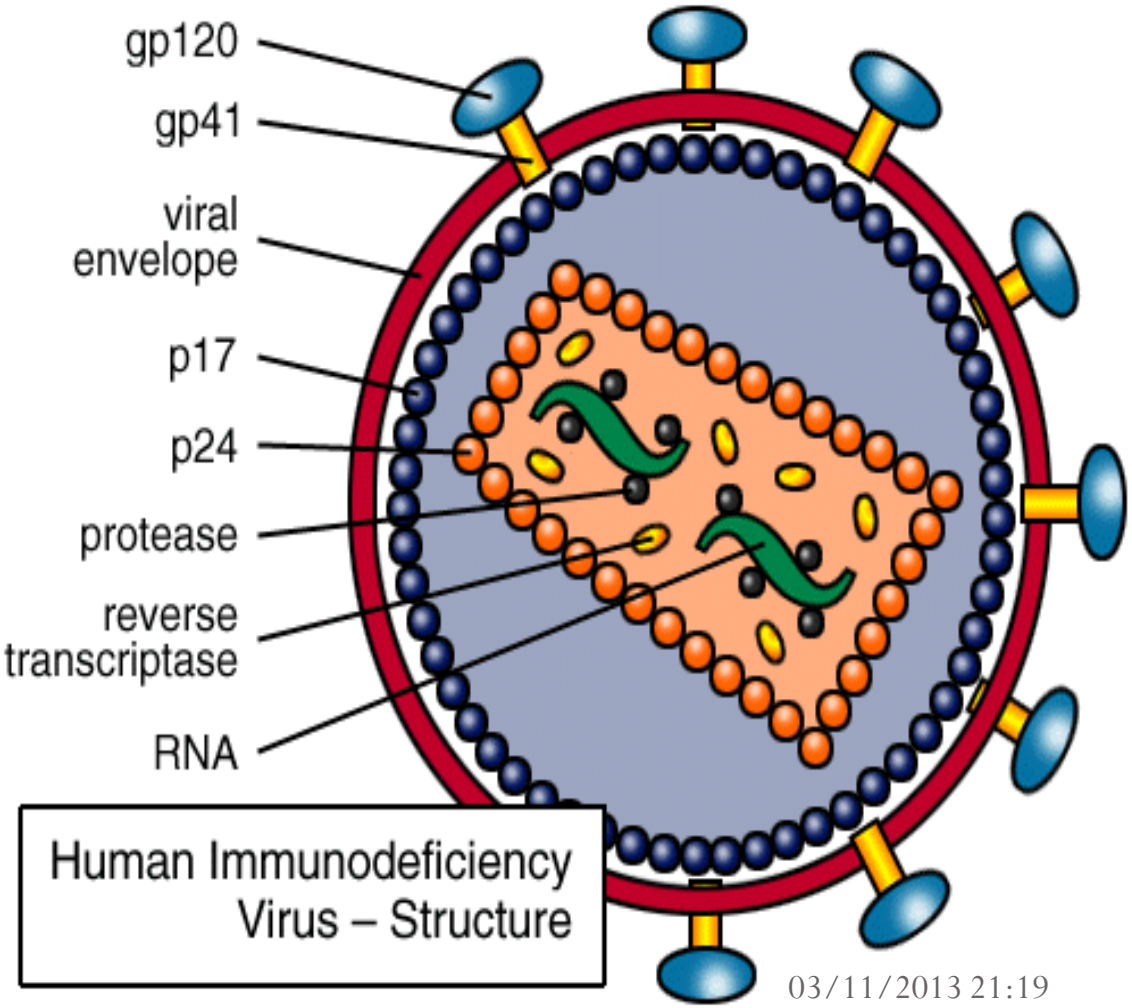
HIV-1 Subtypes

- HIV-1 has many subtypes: A-K
- A-E are the predominant subtypes
  - A: **W. Africa, E. Africa, Central Africa** East Europe & Middle East
  - B: N. America, Europe, Middle East, E. Asia, Latin America
  - C: **S. Africa**, S. Asia, **Ethiopia**
  - D: **E. Africa**
  - E: S. E. Asia

- Unlike other retroviruses, which bud from the infected cell, HIV can lie dormant in the proviral form within a cell for many years, especially in resting (memory) $CD4^+$ T lymphocytes, and may set up a lifelong infection.
- When these cells become reactivated, viral production occurs again and ultimately destroys the cell. Although HIV may disappear from the cells of the circulation, replication and budding continue to occur in other tissues in the absence of chemotherapy

Structure Of HIV

- Has an outer double lipid membrane, (derived from the host membrane).
- The lipid membrane is lined by a matrix protein.
- The lipid membrane is studded with the surface glycoprotein (gp) 120 and the transmembrane gp 41 protein.
- These glycoprotein spikes surround the cone-shaped protein core.



Human Immunodeficiency Virus – Structure

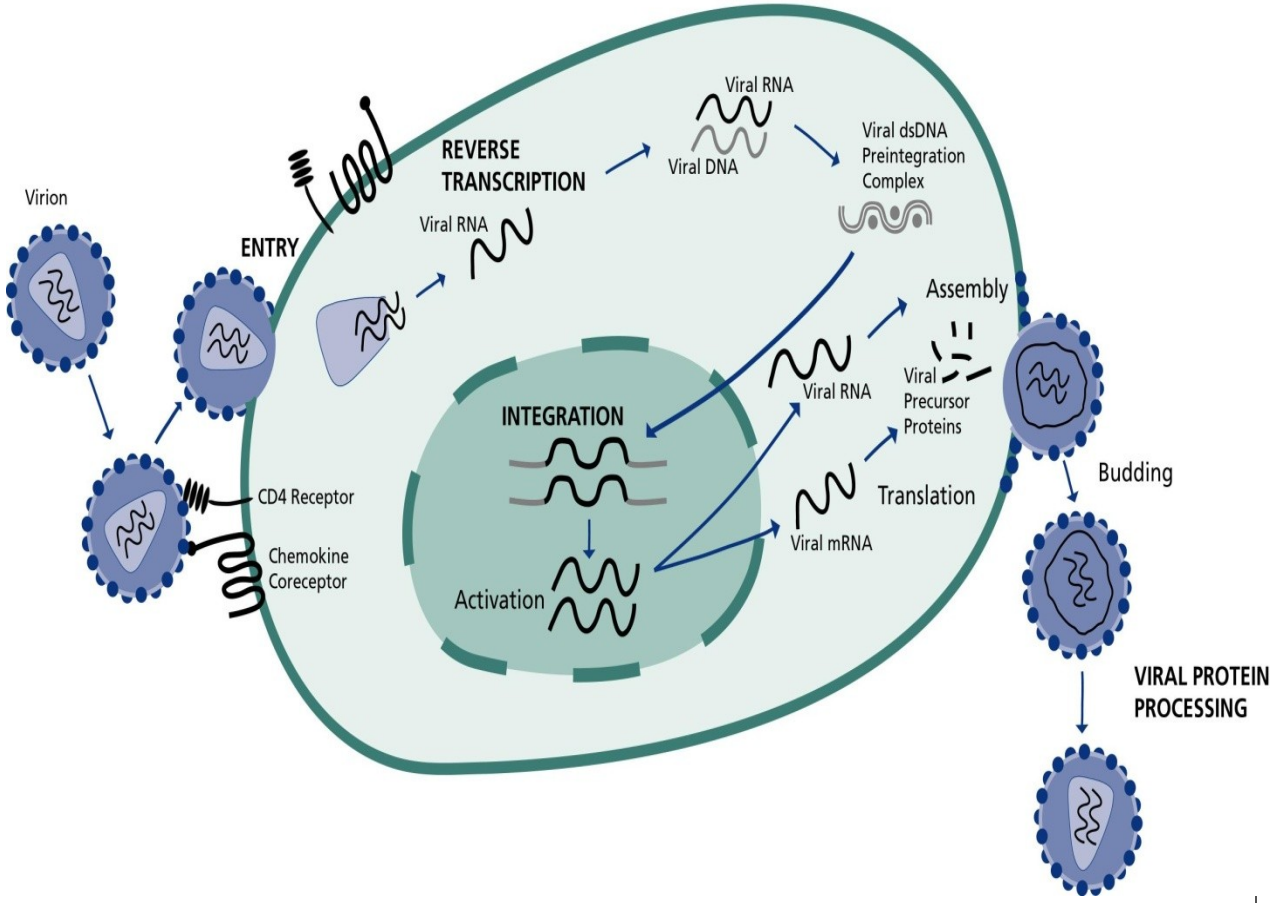
HIV Structure

Viral Enzymes

- Most important: Reverse Transcriptase (RT), Protease and Integrase.
- RT converts viral single-stranded RNA into a double stranded deoxyribonucleic acid (DNA).
- DNA is incorporated into host nucleus as the proviral DNA.
- Integrase facilitates integration of the DNA into the host's chromosomal DNA.
- Protease enzyme splits generated macro-proteins into smaller viral proteins (core, envelope & regulatory proteins and enzymes) which go into forming new viral particles.

HIV Life Cycle

- Binding, Fusion and Entry
- Transcription
- Integration & Replication
- Budding
- Maturation



BINDING:

- For successful entry into cells the HIV envelope glycoprotein GP 120 binds to the host receptor CD4 molecule

FUSION and ENTRY:

- Viral binding to host cell triggers fusion of the viral and host cell membranes
  - Mediated by gp41
- Allows entry of virus core into host cell cytoplasm
- Core protein dissolved by host enzymes releasing viral RNA and enzymes

INTEGRATION

- Reverse transcriptase converts the viral RNA into a DNA molecule
- The DNA enters the host cell nucleus
- **Integrase** catalyses the process of integration of the viral DNA into the host cell's DNA

REPLICATION

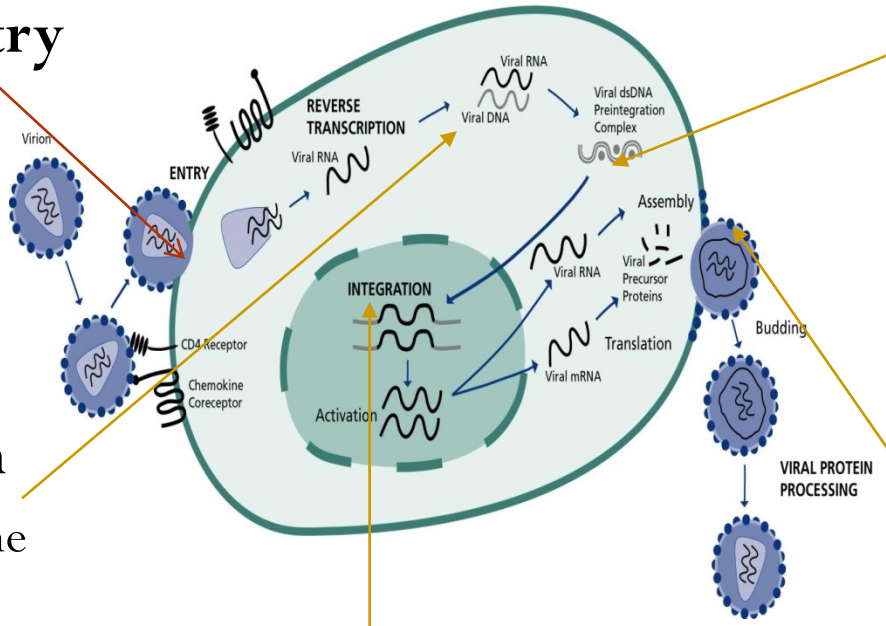
- Integrated viral DNA turns the host cell into a "factory" for manufacturing more virus.
- Viral proteins are produced as a single multi-protein molecule
- Viral proteins cleaved by protease enzyme

Budding and Maturation:

- Viral proteins together with RNA gather at the membrane of the CD4+ cells
- Viral particles are formed which bud off the cell and enter the bloodstream
- The CD4 cells are often destroyed by HIV virus infection and replication resulting in profound immunodeficiency.

HIV LIFE CYCLE: Enzymes

Fusion & entry



- **Reverse Transcription**
  - The viral enzyme **reverse transcriptase** converts the single stranded viral RNA into double strand DNA

Integration:

The viral enzyme **integrase** inserts the viral DNA (viral genetic material) into the host DNA.

- **Transcription:**
  - Activation of host cell results in transcription of viral DNA into mRNA.
  - mRNA translated into viral precursor proteins
- **Assembly & Budding**
  - Viral precursor proteins processed by **protease** enzyme into usable forms
    - Proteins assembled with RNA to form viral particles which then bud

- To show the video clips of viral replication and targets of ARVs

Global summary of the AIDS epidemic 2009

Number of people living with HIV	Total	33.3 million [31.4 million–35.3 million]
	Adults	30.8 million [29.2 million–32.6 million]
	Women	15.9 million [14.8 million–17.2 million]
	Children (<15 years)	2.5 million [1.6 million–3.4 million]
People newly infected with HIV in 2009	Total	2.2 million [2.0 million–2.4 million]
	Adults	370 000 [230 000–510 000]
	Children (<15 years)	1.8 million [1.6 million–2.1 million]
AIDS deaths in 2009	Total	1.6 million [1.4 million–1.8 million]
	Adults	260 000 [150 000–360 000]
	Children (<15 years)	

Impact of HIV in Kenya

- National HIV prevalence is 6.3% based on Kenya Demographic Health Survey (KDHS 2008/9)
- 1.45 m Kenyans estimated to be HIV positive and about 650, 000 need antiretroviral therapy (ART)
- Since 1984 Kenya has experienced a negative impact on all sectors of society as a result of AIDS epidemic.
- Reversed previous health gains: life expectancy reduced

Modes of Transmission

Sexual contact

- In Africa mainly heterosexual
- Homosexual
- Non-consensual sexual exposure (rape)

Parenteral

- Transfusion of infected blood or blood products
- Exposure to infected blood or body fluids through contaminated sharps, needle-sharing or needle stick accidents
- Donated organs, Traditional procedures

Perinatal

- Transplacental, during labor/delivery and breastfeeding

Percent infection by transmission route....

Transmission route	%
Sexual intercourse	**70-80**
Mother-to-child-transmission	**5-10**
Blood transfusion	**3-5**
Injecting drug use	**5-10**
Health care – e.g. needle stick injury	**<0.01**

Biological Factors Influencing HIV Transmission

- **Disease status of source patient**
  - Degree of immunosuppression and viral load - High risk during primary infection and late disease when viral load high
- **Presence of untreated STIs in source and person at risk**
  - Both ulcerative and non ulcerative STIs important cofactors - Related to high viral load in genital secretions during STIs and the disturbance of the genital mucosa
  - A major reason for high prevalence in Sub Saharan Africa

- **Circumcision status**
  - Uncircumcised men 2x as likely to acquire HIV infection than circumcised. Also more likely to acquire STIs

- **Gender differences in susceptibility**
  - Female genital anatomy presents a larger surface area with more of the cells that HIV requires to gain entry
  - (Socio-cultural factors)

- **Host genetic differences**

Socio-economic Factors

- **Social Mobility**
  - HIV/AIDS follows routes of commerce
  - Partners living apart
- **Stigma and Denial**
  - Denial and silence is the norm
  - Stigma prevents acknowledgment of problem and care-seeking
- **People in Conflict**
  - Context of war and struggle of power spreads AIDS
- **Cultural Factors**

Socio-economic Factors (cont'd)

- **Gender**
  - In many cultures it is accepted for men to have many sexual relationships
  - Many women unable to negotiate condom use
- **Poverty**
  - Lack of information needed to understand and prevent HIV
- **Drug Use and Alcohol Consumption**
  - Impaired judgment
  - Sharing of needles and equipment

Behavioral Factors

- Multiple sexual partners
- Unprotected sexual intercourse
- Large age difference between sexual partners

Factors not associated with risk of transmission

- Insect bites
- Saliva (kissing)
- Sneezing or coughing
- Skin contact (e.g. hugging)
- Shared use of facilities (e.g. toilets)

Cells of the immune system

- Responsible for protecting the body from invading foreign bodies - Found in blood and tissues
- In blood mostly are white blood cells (WBC)
  - Macrophages clearing the body of infected, old or damaged cells
  - Neutrophils attack bacteria
  - Eosinophils attack worms (and mediate allergies)
  - B-lymphocytes make antibodies
  - T-lymphocytes
    - Attack viruses, fungi and some bacteria like mycobacteria
    - T helper (CD4) cells assist in function of other immune cells
    - CD8 or T killer cells are able to destroy infected cells

How HIV Affects Immune System

- HIV attaches to cells of the immune system through special surface markers called CD4 receptors
- The following immune cells have CD4 receptors
  - T-Lymphocytes – CD4+ Cells
  - Macrophages
  - Monocytes
  - Dendritic cells
- HIV infection of CD4 cells causes cell dysfunction and death
- The hallmark of HIV/AIDS is a profound immunodeficiency as a result depletion of CD4+ T lymphocytes.
- The CD4+ T cell depletion is two fold
  - Reduction in numbers
  - Impairment in function

- Reduction in the CD4 cell number and the effects on their function reduces the capacity of the body to fight infectious diseases.
- Individuals with HIV infection are therefore increasingly susceptible to many infections especially at later stages of HIV infection

Primary HIV Infection

- On exposure, there is a 2-4 week period of intense viral replication and widespread dissemination of virus characterized by
  - High plasma viral load (RNA)
  - Rapid decline in CD4 count
  - In some cases an acute illness occurs
    - Lasts from 1-2 weeks, but it is rarely diagnosed
- Symptom resolution with reduction in plasma viremia due to development of an immune response and antibodies to the virus

Asymptomatic Disease (Latency)

- Patients then enter a stage of asymptomatic disease phase lasting on average 2-10 years (clinical latency)
- Characterized by gradual decline in CD4 count
  - Rate depends on viral load
- Host genetic/immunological or viral factors may be involved

Symptomatic Disease and AIDS

- Viral load continues to rise causing
  - Increased demands on immune system as production of CD4 cells cannot match destruction
  - Increased susceptibility to common infections (URTI, pneumonia, skin etc)
  - Late-stage disease is characterized by a CD4 count <200cells/mm^3 and the development of opportunistic infections, selected tumors, wasting, and neurological complications).

Opportunistic Infections

- The predominant causes of morbidity and mortality among patients with late-stage HIV infection are opportunistic infections, ie, severe infections induced by agents that rarely cause serious disease in immune-competent individuals.
- The most common opportunistic infections in untreated AIDS patients include the following:

1.Protozoa: *Toxoplasma gondii, Isospora belli, Cryptosporidium* species.

2. Fungi: *Candida albicans, Cryptococcus neoformans, Coccidioides immitis, Histoplasma capsulatum, Pneumocystis jiroveci.*

Opportunistic Infections

3. Bacteria: *Mycobacterium avium-intracellulare, Mycobacterium tuberculosis, Listeria monocytogenes, Nocardia asteroides, Salmonella* species, *Streptococcus* species.

4. Viruses: Cytomegalovirus, herpes simplex virus, varicella-zoster virus, adenovirus, polyomavirus, JC virus, hepatitis B virus, hepatitis C virus.

- Herpesvirus infections are common in AIDS patients, and multiple herpesviruses are frequently detected being shed in saliva. Cytomegalovirus retinitis is the most common severe ocular complication of AIDS.
- Cancer

Laboratory diagnosis

- **Serological methods: antibody/antigen based test**
  - Enzyme Immunosorbent Assay (ELISA)
  - Western Blot
- **Viral detection methods**
  - PCR
  - Culture: Rarely used

Serological methods

- Common and widely used tests
- Based on the principal of antigen antibody reaction
- Antibody takes some time to be produced after an infection
- Therefore all these tests have a problem of making a diagnosis during the period immediately after infection with HIV prior to the appearance of detectable antibodies

Rapid Tests

- These are ELISA tests
- Can be performed in less than 20 minutes
  - therefore also referred as "Simple/Rapid" (S/R) assays
- Can be performed easily without instruments
- A positive result is indicated by the appearance of a colored dot or line
- Examples – Bioline, Determine, Unigold and Oraquick.

Limitations of the HIV test

- A negative result doesn't eliminate the possibility of HIV 1/HIV 2 infection.
- The specimen may contain low levels of antibodies to HIV 1/HIV 2.
- Diagnosis is based on more than one kit

1. PICORNAVIRUSES

- Picornaviruses are the small RNA
- Have a naked nucleocapsid that is about 30mm in diameter.
- Pico means *small*, hence small RNA viruses or
- There are nine genera within the*Picornaviridae*. Five of these infect humans:
  - Enteroviruses
  - Rhinoviruses
  - Hepatoviruses
  - Parechoviruses
  - Kobuviruses

Enterovirus **Polio** **Coxsackie A and B** **Echo** **Other enteroviruses**	**Diseases of the human (and other) alimentary tract (e.g. polio virus)**
Rhinovirus	Disease of the nasopharyngeal region (e.g. common cold virus)
Hepatovirus	Human hepatitis virus A
Parechovirus	Formerly echoviruses 22 and 23. Disease of alimentary and respiratory tract
Kobuvirus	Aichi virus is the type species

ENTEROVIRUSES

- Spread via the fecal-oral route. The ingested viruses infect cells of the oro-pharyngeal mucosa and lymphoid tissue (tonsils) where they are replicated and shed into the alimentary tract
- Pass into the intestine and set up further infections in the intestinal mucosa. The virus also infects the lymphoid tissue (Peyer's patches)
- The virus replicates and are shed into the feces, often for months after the primary infection

POLIOVIRUS

- Poliomyelitis means inflammation of the gray (*poliós*) spinal cord (*myelós)*. It is also known as infantile paralysis.
- First recorded case of poliomyelitis comes from an Egypt (1580-1350 BCE)
- There are three serotypes of polio virus. Most disease results from type 1 polio virus.
- Since the disease is spread by fecal contamination, infections are more common where unsanitary conditions prevail but many children in these areas have asymptomatic infections that lead to life-long immunity.

Asymptomatic polio infection

- In more than 90% of the cases, infection is asymptomatic. This occurs when the replication of the virus is restricted to the GIT
- Why many polio infections are asymptomatic may be due to; the size of the innoculum of the virus, the size of the resulting viremia, the virulence of the infecting virus, and the presence of circulating antibodies.

Abortive poliomyelitis (minor illness)

- The first symptomatic result of polio infection is febrile disease and occurs in the first week of infection. The patient may exhibit a general malaise which may be accompanied by vomiting, a headache and sore throat. This is abortive poliomyelitis and occurs in about 5% of infected individuals

Non-paralytic poliomyelitis

- Three or four days later a stiff neck and vomiting, as a result of muscle spasms, may occur in about 2% of patients. This is similar to aseptic meningitis. The virus has now progressed to the brain and infected the meninges.

Paralytic polio

- About 4 days after the end of the first minor symptoms, the virus has spread from the blood to the anterior horn cells of the spinal cord and to the motor cortex of the brain. The degree of paralysis depends on the which neurons are affected and the amount of damage that they sustain. The disease is more pronounced in very young and very old patients

- In spinal paralysis one or more limbs may be affected or complete flaccid paralysis may occur.
- In bulbar paralysis cranial nerves and the respiratory center in the medulla are affected leading to paralysis of neck and respiratory muscles.
- The degree of paralysis may increase over a period of a few days and may remain for life or there may be complete recovery over period of 6 months to a few years.

Post-polio syndrome

- This afflicts victims of an earlier polio virus infection but the virus is no longer present. It may occur many years after the infection and involves loss of function in affected muscles, perhaps as a result of further neuron loss.

COXSACKIE VIRUSES

- Coxsackie type A usually is associated with surface rashes (exanthems) while type B typically causes internal symptoms (eg myocarditis) but both can also cause paralytic disease or mild respiratory tract infection.
- **Meningitis**
- Enteroviruses are the major cause of viral meningitis. Both Coxsackie virus A and B can cause aseptic meningitis which is so-called because it is not of bacterial origin. Viral meningitis typically involves a headache, stiff neck, fever and general malaise.

PREVENTION OF PICORNAVIRUS DISEASE

- Vaccination is the major means of control of this virus
- There are no vaccines for Coxsackie virus or other enteroviruses

RHINOVIRUSES

- Rhinoviruses are one of the families of viruses that can cause the common cold although many other viruses can infect the respiratory tract and cause cold-like symptoms.
- About one third of "colds" are caused by rhinovirus infections. There are more than 100 serotypes explaining why vaccines against rhinoviruses have proved difficult to develop.
- Are spread by aerosols and infect the upper respiratory tract, can also be spread by fomites such as hands and other forms of direct contact.
- Are sensitive to temperature. Thus, they do not spread to the lower respiratory tract since they replicate best at a few degrees below normal body temperature.

RHINOVIRUS DISEASE

- There are nearly 62 million cases of the common cold annually in the US
- 52.2 million of these cases affect Americans under age 17
- There are nearly 22 million school-loss days annually due to the common cold
- There are approximately 45 million bed days annually associated with the common cold
- Seventy-five percent of common colds suffered by children under 5 years are medically attended
  Source: *Vital and Health Statistics Series 10, No. 200*
- The symptoms of a rhinovirus infection are well known: discharging or blocked nasal passages often accompanied by sneezes, and perhaps a sore throat. This typical "runny nose" (rhinorhea) may be accompanied by a general malaise, cough, sore throat etc. The characteristic