Ministry of Higher Education & Scientific Research
Al-furat Al-awsat Technical University
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Medical microbiology

By

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For

Dialysis Students

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Lecture 1 :

What are the purposes of the study of medical microbiology ?

The purposes of the study of medical microbiology are

1. To work with clinician and other health team members to provide diagnosis of infectious disease in patients.
2. To prevent the spread of infectious to other peoples.
3. To provide suitable therapy early in the course of illness.
4. To determine the appropriate and effective antibiotic for treatment to avoid hospitalization ,mortality and morbidity.

A-Biosafety & Biosecurity:-

Biosafety: The containment principles, technologies and practices that are implemented to prevent the unintentional or intentional exposure to pathogens and toxins, or their accidental release. The maintenance of safe conditions in biological research to prevent harm to workers, nonlaboratory organisms, or the environment.

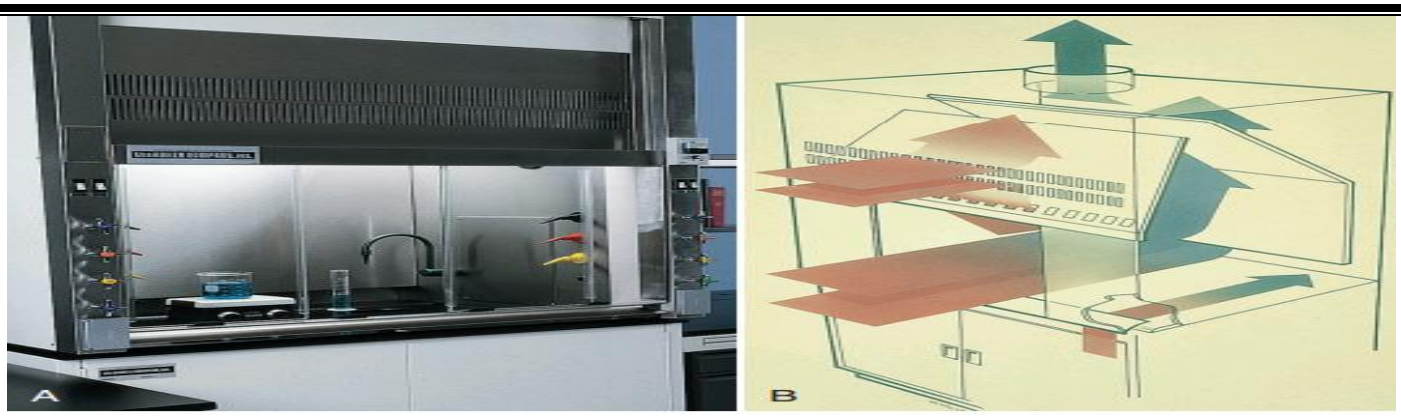
Biosecurity: -biosecurity" will refer to the protection of microbial agents from loss, theft, diversion or intentional misuse.

B-laboratory safety:

General consideration:

Microbiology laboratories should contain safety risks such as fire ,electrical hazards, chemical hazards, slippery floor and radioactive materials.

Biohazards : Biological safety cabinet (BSC): It is an advice that encloses the work space to protect workers from aerosol exposure to infectious disease. Air that contains the infectious materials is sterilized by heat, Uv-light or by passage through a high-efficiency particulate air filter that removes most particles larger than 0.3Mm in diameter .



Biological safety cabinet

Biosafety levels :

1. Biosafety level 1 (**BSL-1**) agents: These agents have no known pathogenicity (non-pathogenic) which are useful in lab. Teaching exercises for students of microbiology. These agents include *Bacillus subtilis* , *Staphylococcus epidermidis* and other non- pathogenic organisms.
2. Biosafety level 2 (**BSL-2**) agents: These include all of the common agents of infectious diseases such as HIV, Hepatitis B virus, , *Salmonella spp*, *Shigella spp*, and other infectious organisms.
3. Biosafety level 3(**BSL-3**) : procedures have been recommended for the handling of material suspected of harboring organisms unlikely to be encountered in a routine clinical laboratory and for such organisms as *Mycobacterium tuberculosis*, *Coxiella burnetii*, and the mold stages of systemic fungi and for some other organisms when grown in quantities greater than that found in patient specimens.
4. Biosafety level 4 (**BSL-4**): These agents considered high risk and cause life-threatening disease include only certain viruses of Abroviruses or Filoviruses groups. Working with such viruses requires the use of maximum facilities. Personal and all materials are decontaminated before leaving the lab. and use special protective cloths and cabinet.

Personal protective equipment (PPE): is the first line of defense for laboratory personnel working with microbiological agents. PPE creates a barrier between the user and potential hazards, significantly reducing the risk of exposure to infectious materials

PPE Item	Function	Considerations
Gloves	Protect hands from microbes and minimizes the possible spreading of microbes	Material compatibility, dexterity
Lab Coat: Includes gowns, aprons, head covering, and shoe covers	Protect skin and clothing	Fluid resistance, comfort
Eye Protection includes face shields as well as goggles	Prevent splashes to eyes They can protect the mucous membranes of your eyes from bodily fluids.	Fog resistance, fit over prescription glasses
Masks:	Protect from airborne particles: Cover your mouth and nasal area. Some masks have a see-through plastic piece, which also covers your eyes (shield). A special respiratory mask (respirator) forms a tight seal around your nose and mouth. It can be useful to minimize the inhalation of smaller microbes, such as tuberculosis bacteria	Proper fit, appropriate filter type

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Lecture 2 :

Microbiology: is the science deals with the study of the biology of microscopic organisms (microorganisms), this includes bacteria, viruses, fungi, protozoa and algae .

Microorganisms: living forms too small , can be seen under microscope which s that exist as single cells or cell clusters; it also includes viruses, which are microscopic but not cellular .

Classification of microorganisms:

Microorganisms of medical importance can be classified :

1. **Prokaryotic cell**	2. **Eukaryotic cell**
Simple structure	Complex structure
No prominent nucleus (no nuclear membrane	Prominent nucleus (has nucleus membrane)
Small size	Large size
Cell wall has peptidoglycan	Cell wall has chitin or cellulose
Small ribosome	Large ribosome
Single chromosome present	More than one chromosome present
Unicellular	Unicellular or multicellular
Organelles are absent such as (Lysosome, mitochondria , and Golgi apparatus)	organelles are present , such as (mitochondria and lysosomes, and Golgi apparatus
Example: Bacteria	Example: Animal, plant, fungi and protista

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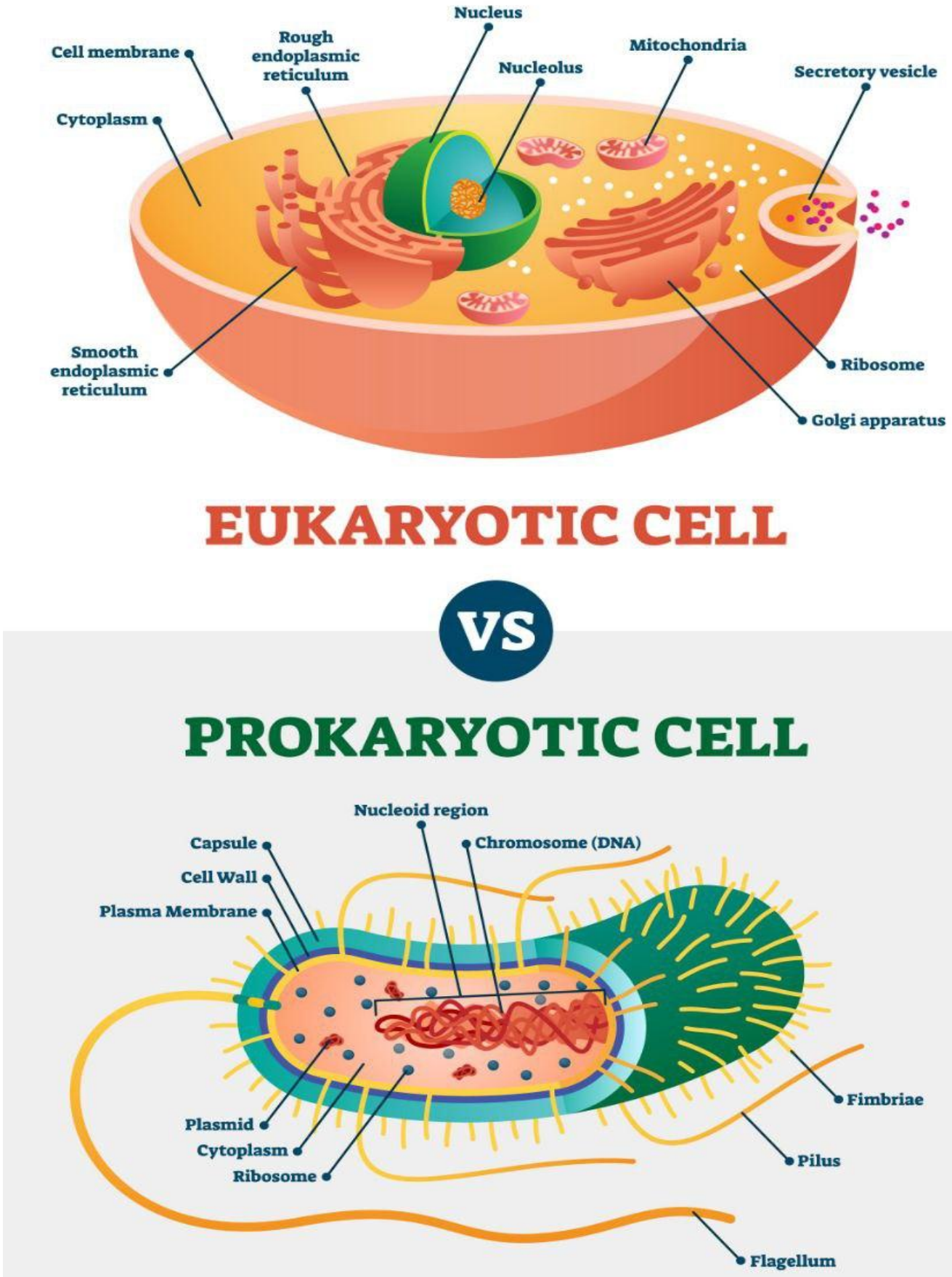


Figure : Difference between prokaryotic and eukaryotic cell

3. <u>Viruses</u>:-

1. Are not cells and are not visible with the light microscope.
2. Are obligate intracellular parasites.
3. Contain no organelles or biosynthetic machinery, except for a few enzymes.
4. Contain either RNA or DNA as genetic material.
5. Are called bacteriophages (or phages) if they have a bacterial host

Viral disease is some harmful abnormality that results from viral infection of the host organism. Viruses tend to exhibit organ and cell-type specificities

- Cutaneous Viral Diseases

Clinical Appearance: Warts , rashes, and lesions are common.

Diagnosis: , Physical exam and molecular tests like PCR.

Treatment: Antivirals for specific conditions, or supportive care.

- Genitourinary Viral Diseases

Clinical Appearance: Sexually transmitted infections (STIs) can cause sores, ulcers, or pain in the genital area. Urinary tract infections (UTIs) can cause painful urination and frequency.

Diagnosis: Physical exam, lab tests (like urinalysis and cultures for bacteria), and PCR to identify specific viruses.

Treatment: Antiviral medications for viral STIs like herpes, or supportive care.

- Gastrointestinal Viral Diseases

Clinical Appearance: abdominal cramps ,Watery diarrhea, and vomiting, nausea.

Diagnosis: Stool samples and PCR can identify specific viruses.

Treatment: Supportive care with hydration and rest.

- Hepatic Viral Diseases

Clinical Appearance: abdominal pain, dark urine, and jaundice (yellowing of the skin and eyes).

Diagnosis: Blood tests to check liver function and detect viral

Treatment: Supportive care, and specific antiviral drugs for some types of hepatitis (e.g., Hepatitis C).

- Hemorrhagic Viral Diseases

Clinical Appearance: Fever, with severe cases developing shock and bleeding (hemorrhage).

Diagnosis: viral isolation, PCR, , and serological testing.

Treatment: Supportive care is essential, including fluid replacement and shock management. Specific antiviral drugs are available for some viruses.

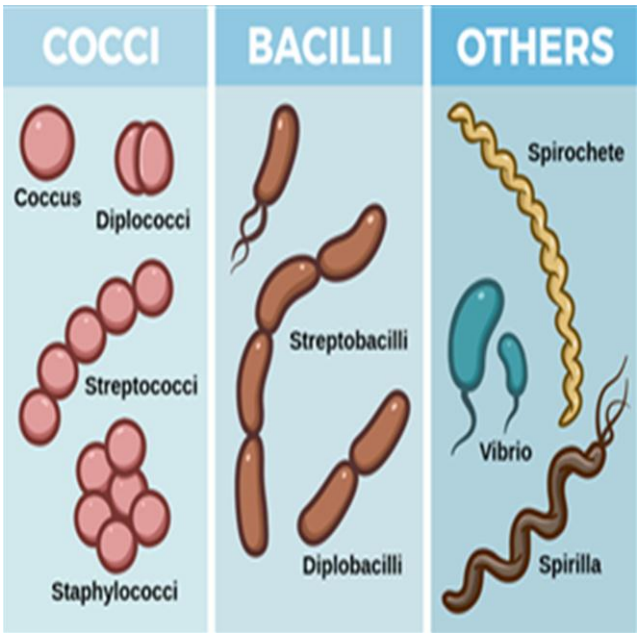
Lecture 3:

General introduction of bacteriology

Shape & size of bacteria

The shape of a bacterium is determined by its rigid cell wall. The microscopic appearance of a bacterium is one of the most important criteria used in its identification. bacteria are classified by shape into three basic groups:

1. **Cocci** : are round oval or spherical cells. These may be arranged in
   - pairs (diplococci)
   - chains (streptococci)
   - grapelike clusters (staphylococci)



2. **Bacilli**: The bacilli are rod shaped.

   These bacilli may show either of the following arrangement:

   - Coccobacilli: Length of the bacteria is approximately the same as its width.
   - Streptobacilli: These are arranged in chains.
   - Comma shaped: They exhibit curved appearance.
   - Spirilla: They exhibit rigid spiral forms.

3. **Spirochetes** : Spirochetes are **slender**, **flexuous spiral** forms.

Size of most bacteria ranges from **1 to 3 µm**. Mycoplasma, the smallest bacteria (and therefore the smallest cells), are **0.2 µm**. Some bacteria, are as long as **10 µm**; that is, they are longer than a human red blood cell, which is 7 µm in diameter.

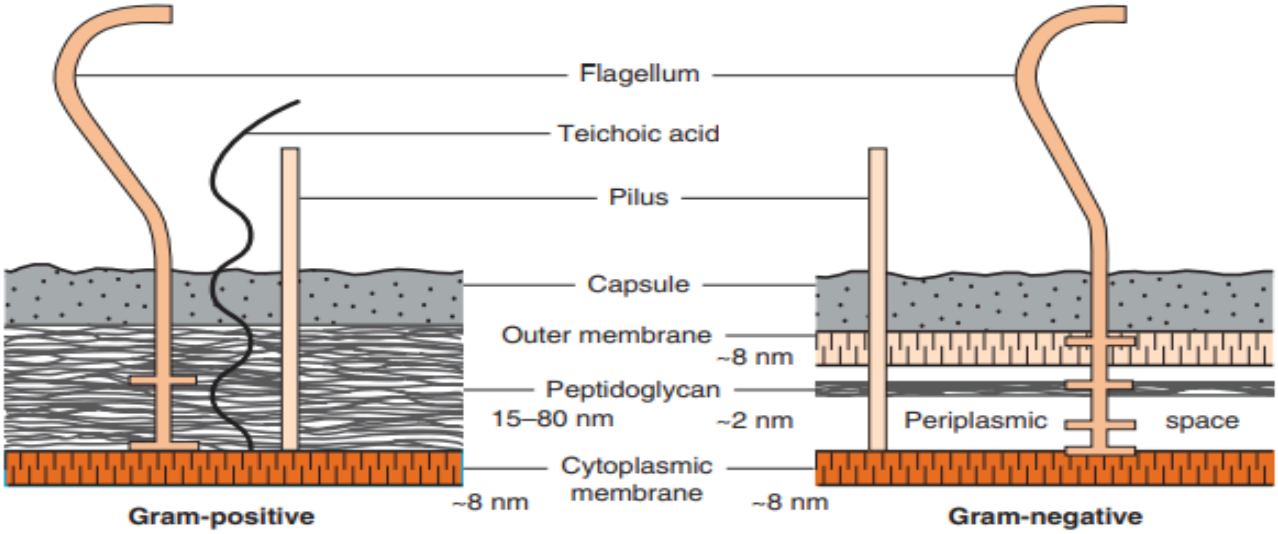
Bacterial cell structure

1- Essential components

- **Cell wall**: Peptidoglycan is the main component of the cell wall and Gives shape and rigid support, protects against osmotic pressure of the cell. Bacterial cells can be classified into **Gram-positive** or **Gram-negative** based on the structural differences between Gram-positive and Gram-negative cell walls.

Gram positive and negative cell wall structure

Component	Gram-Positive Cells	Gram-Negative Cells
Cell wall	simple	More complex
Peptidoglycan	Thicker; multilayer	Thinner; single layer
Teichoic acids	Presence	Absence
Lipopolysaccharide (endotoxin)	Absence	Presence
Susceptible to antibiotics	More susceptible	More resistant



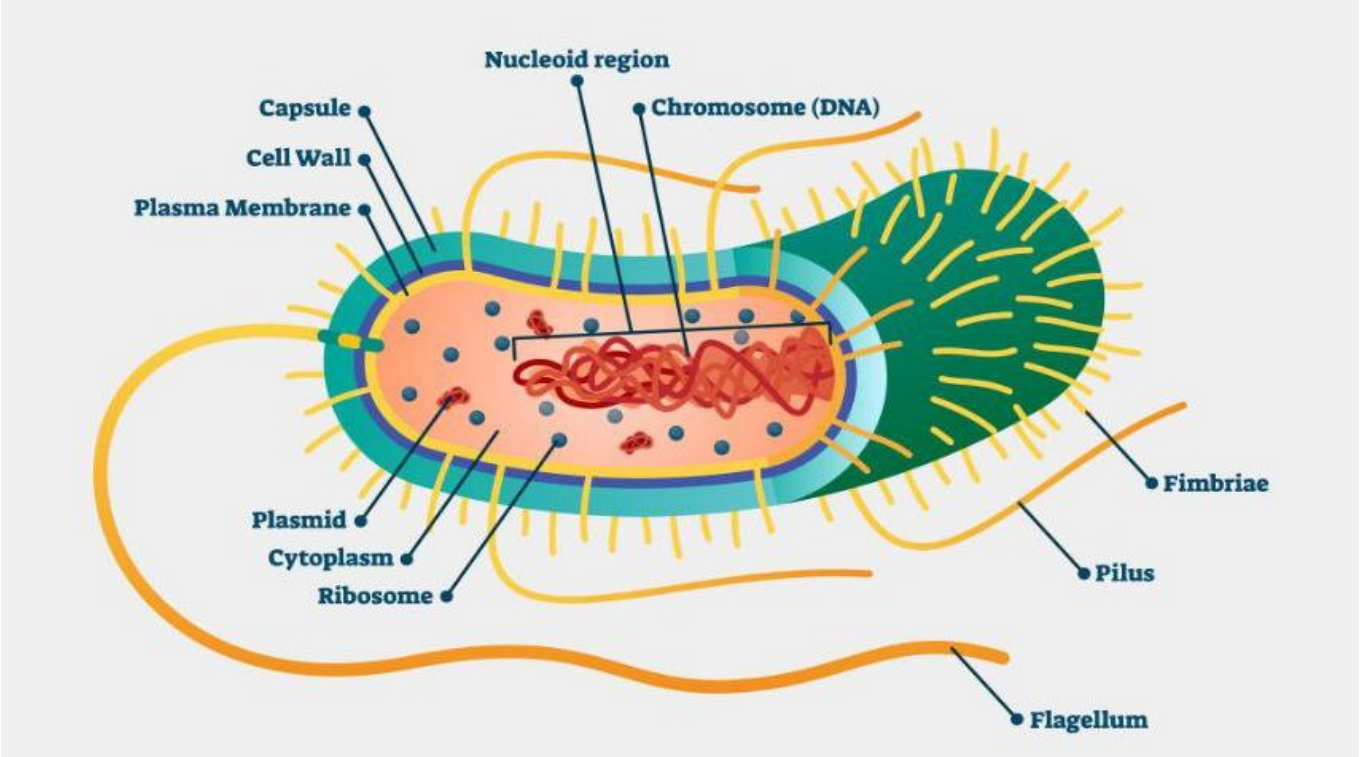
Cell walls of gram-positive and gram-negative bacteria.

- **Cytoplasm:** is a gel containing cellular organelles.
- **Cell membrane or plasma membrane**: , its **composed of** phospholipids about 40% and protein 60% it's found in prokaryotic cell . It acts as a semipermeable membrane regulating the inflow and outflow of metabolites to and from the protoplasm.
- **Ribosomes:** its composed of RNA and protein, they serve as the sites of protein synthesis.
- **Nucleoid or Chromosomal DNA**: prokaryotic organisms have a single, circular chromosome(**genetic material**) .
- **Periplasm** : is a space between plasma membrane and outer membrane Contains many hydrolytic enzymes

2- Nonessential components

- **Capsule**: is a polysaccharide gel-like layer outside the cell wall, the capsule has various functions:
  - Protecting the bacteria from phagocytosis.
  - Prevents bacteria from generating immune response in infected hosts.
  - It facilitates adherence of bacteria to surfaces
  - Play a role in resistance to desiccation.

- **Flagella**: are long, whip like appendages that move the bacteria toward nutrients and other attractants. These structures are responsible for conferring motility to the bacteria.
- **Pili (fimbriae**): Pili are hair like filaments that extend from the cell surface. They are shorter and straighter than flagella, They are found mainly on gram-negative organisms. Pili play a major role in the adherence of bacteria to host cells and transfer of bacterial DNA takes place through sex pili during the process of conjugation.
- **Plasmid**: are extra chromosomal, double-stranded, circular DNA molecules that are capable of replicating independently, they code for many different functions and structures, for example antibiotic resistance.



Structure of bacteria

Sterilization and disinfection , Culture media and staining

- **Sterilization**: is the **killing of all forms of microbial life**, including bacterial spores. Spores are resistant to boiling, so sterilization of medical equipment is typically achieved at 121°C for 15 minutes in an autoclave. Sterilization of heat-sensitive materials is achieved by exposure to ethylene oxide, and liquids can be sterilized by filtration.
- **Disinfection** : is **reducing the number of bacteria** to a level low enough that disease is unlikely to occur. Spores and some bacteria will survive. For example, disinfection of the water supply is achieved by treatment with chlorine.

Antiseptics are disinfectants that are mild enough to use on skin and other tissues, such as 70% ethanol.

The killing of microbes by either **chemicals** or **physical** agents

- **Chemical** agents: like (alcohols , detergents, Phenols, Chlorine, Iodine, Hydrogen Peroxide, Formaldehyde , Glutaraldehyde, and Ethylene Oxide)
- **physical** agents: like (Heat ,radiation ,and filtrations)

Culture media and staining

In the laboratory, the nutrients are incorporated into culture media on or in which bacteria are grown. If a culture medium meets a bacterial cell's growth requirements, then that cell will multiply to sufficient numbers to allow visualization by the unaided eye.

Classification of culture media:

1- **Based on their consistency**

- Solid medium
- Liquid medium
- Semi solid medium)

2. **Based on the constituents /ingredients**

- Simple medium

- Complex medium
- Special medium :
  - (i) Enrichment media
  - (ii) Enriched Media
  - (iii) Selective media
  - (iv) Differential media
  - (v) Transport media

3. **Based on oxygen requirement**

- Aerobic media
- Anaerobic media

Bacterial staining is the process of coloring of colorless bacterial structural components using stains (dyes). The principle of staining is to identify microorganisms selectively by using dyes.

Types of staining:

- **Simple stain** : (methylene blue, and crystal violet)
- **Deferential stain** :(Gram stain, Acid fast stain)
- **Special staining** :(Capsule , spore , and India ink stain)

Lecture 4:

Environmental conditions are affected on the growth of bacteria

Bacteria must be able to respond to variations in nutrient levels, particularly for nutrient limitation. Growth of bacteria also affected by the chemical and physical factors for their surrounding environment. Most important environmental factors that affect microbial growth are:

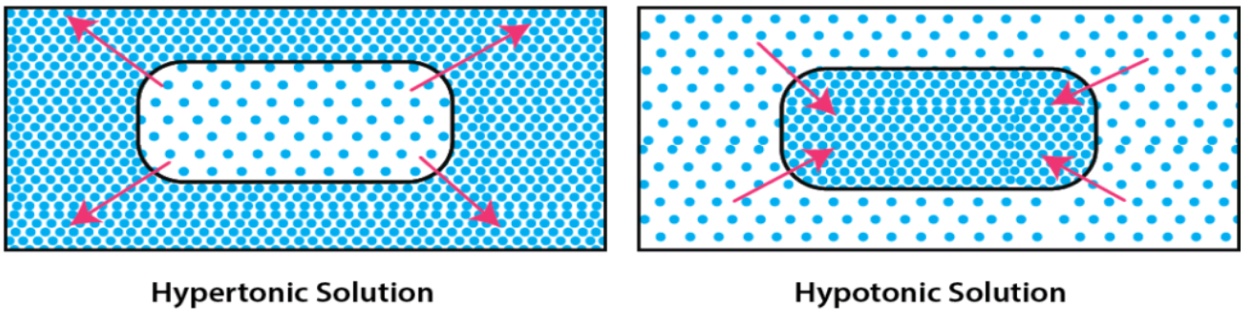
- **Osmolarity** :

Cells are subject to changes in **osmotic pressure**

bacteria grow best in neutral **(Isotonic)** environment (**0.8 – 0.9 % NaCl**) .

If the concentration of NaCl is increasing in the environment, the cell membrane will be damaged (shrinkage) and stops the bacterial growth (**Hypertonic**).

If the concentration of NaCl is decreasing in the environment, the cell membrane will be burst(swelling) and stops the bacterial growth (**Hypotonic**)



Hypertonic Solution

Hypotonic Solution

- **pH**

The optimal pH must be empirically determined for each species.

- **Neutralophiles**: most organisms grow best at a **pH of 6.0–8.0**.
- **Acidophiles**: have optima as **low as pH 3.0**.
- **Alkaliphiles** : have optima as **high as pH 10.5** ex *Vibirio cholera*

- **Moisture :** water is essential for the growth of bacteria .
- **Light:** all organisms grow best in the darkness .
- **Temperature :** different microbial species vary widely in their optimal temperature ranges for growth

the temperature at which an organism grows best called **optimum temperature .**

in human parasitic organism the optimum temperature range between **30 – 37 °C** .

There are three groups of bacteria as regards to temperature of growth :

- **Psychrophilic bacteria : grow best at low temperatures (–5 to 15°C)**
- **Mesophilic bacteria** : grow best at (**30–37°C),** include bacteria production disease

❖ **Thermophilic bacteria : grow best at 50–60°C**

➢ **Aeration:** bacteria can be classified according to its need to **oxygen** :

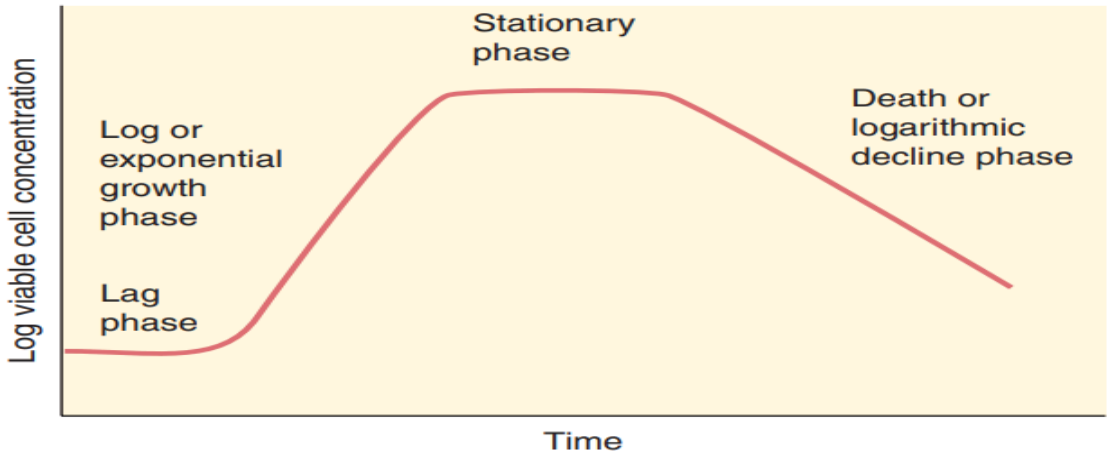
❖ **Obligate aerobes :** requiring oxygen to grow .

❖ **Obligate anaerobes :** cannot grow in the presence of oxygen .

❖ **Facultative aerobes :** able to live aerobically or anaerobically.

Stages of bacterial growth

Bacteria reproduce by **binary fission**, a process by which one parent cell divides to form two progeny cells. Because one cell gives rise to two progeny cells. The growth cycle of bacteria has **four major phases**. If a small number of bacteria are inoculated into a liquid nutrient medium and the bacteria are counted at frequent intervals, the typical phases of a standard growth curve can be demonstrated in below figure :



➢ **Lag phase**: during which strong metabolic activity occurs but cells do **not divide**. This can last for a **few minutes up to many hours**.

➢ **Log (logarithmic) phase**: is characterized by rapid exponential cell growth (i.e., 1 to 2 to 4 to 8 and so on). In this phase, the microbes are sensitive to adverse conditions, such as antibiotics and other antimicrobial agents.

➢ **Stationary phase**: the bacterial growth almost stops completely due to lack of essential nutrients, lack of water oxygen, change in pH of the medium, and toxic products cause growth to slow until the number of new cells produced balances the number of cells that die.

➢ **Decline phase**: decline in the number of viable bacteria until all organism die.

Bacterial growth inhibition

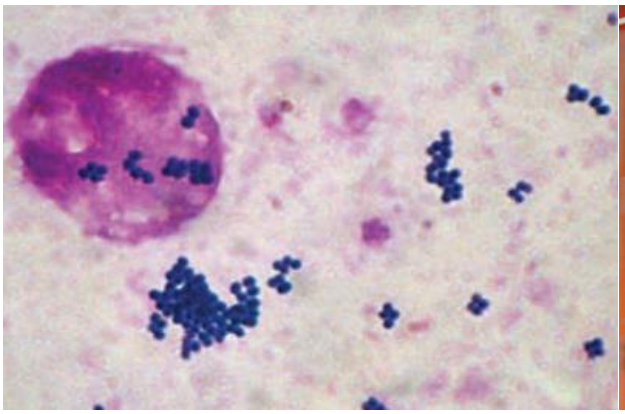
- Nature and source of **nutrient** for the bacterial growth on
- The **pH** of the feed material
- **Temperature** and moisture levels will also influence the magnitude of growth.
- **Oxygen** and **time** requirement for the bacterial growth on
- Concentration of **antibiotic** product and **waste**

Lecture 5:

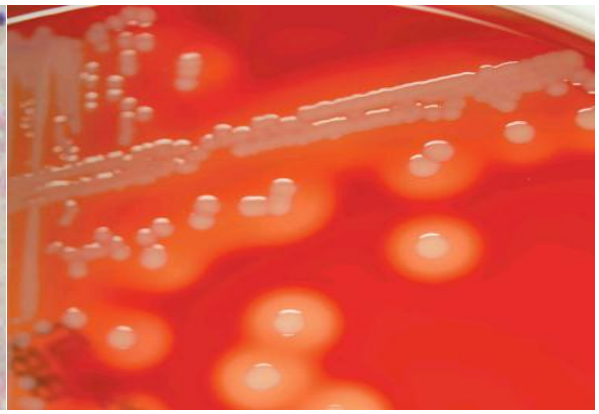
Medical important bacteria

Staphylococci Group

- ❖ Staphylococci generally stain darkly gram positive
- ❖ Staphylococci are round rather than oval and tend to occur in bunches like grapes, facultative anaerobic organisms, non-spore forming ,and non-motile
- ❖ cultured on enriched media containing nutrient broth and/or blood , *S. aureus* produce β- hemolysis .
- ❖ Catalase positive .
- ❖ Resistant to heat and drying, and thus can persist for long periods on fomites (inanimate objects), which can then serve as sources of infection.



Gram stain of *Staphylococcus aureus*



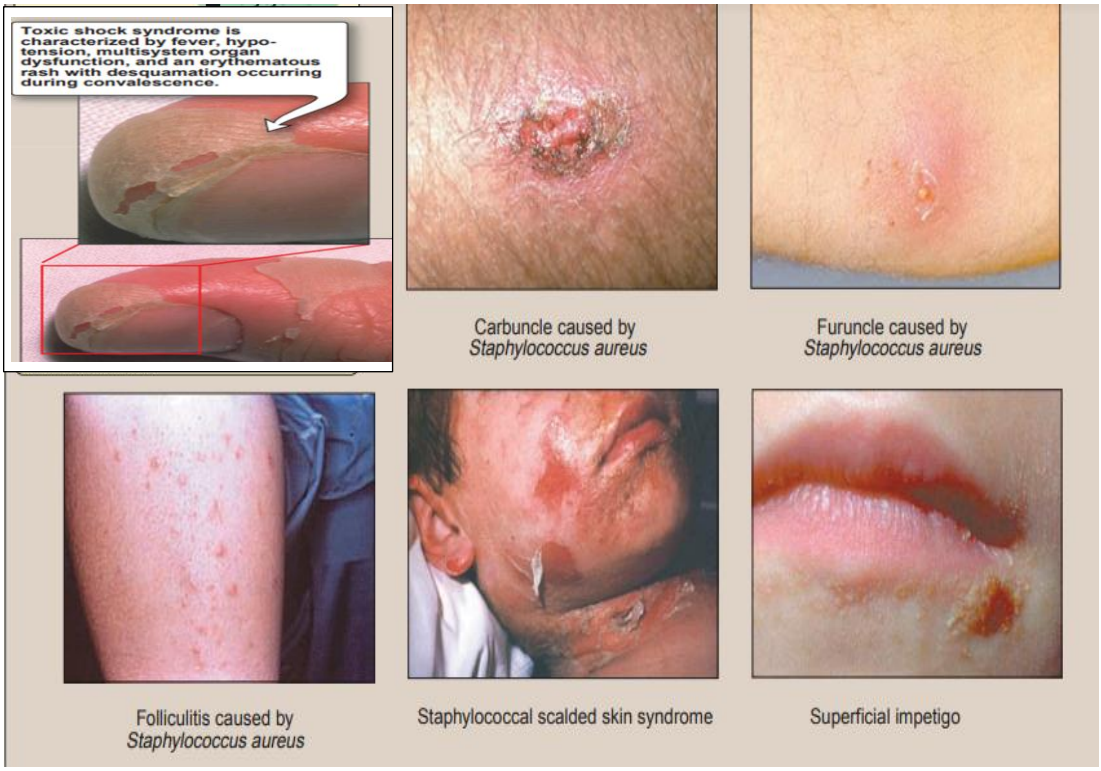
Colonies of *Staphylococcus aureus* on a blood agar

Staphylococcus aureus

- Usual member of the microbiota of the body, carried by healthy individuals on the skin and mucous membranes.
- Act as a commensal of the human microbiota (normal flora) it can also become an opportunistic pathogen.
- Source of infection or contamination of food, which can then result in food poisoning.

➢ **Disease**

- Skin and soft tissue infections
- Necrotizing pneumonia
- Osteomyelitis
- Septic arthritis
- Endocarditis
- Septicemia
- Toxic shock syndrome
- Food poisoning



- **Virulence factors:**
  - **polysaccharide Capsule :** Inhibits phagocytosis
  - **Protein A**: Protein A is a major component of the *S. aureus* cell wall, act as strongly anti-phagocytic effect.
  - **Enterotoxins** : stimulates the vomiting center in the brain (Food poisoning)
  - **Teichoic acids** : mediate adherence of the staphylococci to mucosal cells.
- **Treatment**

Penicillin , benzyl penicillin , cephalosporin , erethromycin , cloxacillin , clindamycin**.**

Antibiotics resistance:

*** Penicillin resistance in staphylococci**

Nearly 80% or more strains of *S. aureus* are resistant to penicillin. Penicillin-resistant strains can be treated with e.g., methicillin and vancomycin.

*** Methicillin-resistant staphylococci (MRSA)**

Methicillin-resistant S. aureus (MRSA) denotes resistance of *S. aureus* to penicillin, as well as cephalosporins and carbapenems. MRSA strains can be treated with vancomycin.

***Vancomycin- resistant staphylococci**

Strains of *S. aureus* with intermediate resistance to vancomycin (VISA) and with full resistance to vancomycin (VRSA).

Staphylococcus epidermidis

- present in large numbers as part of the normal flora of the skin
- it is frequently recovered from blood cultures, generally as a contaminant from skin.
- Despite its low virulence, it is a common cause of infection of implants such as heart valves and catheters .
- Acquired drug resistance by *S. epidermidis* is even more frequent than by *S. aureus*. Vancomycin sensitivity remains the rule.
- *S. epidermidis* produces an extracellular polysaccharide material called polysaccharide intercellular adhesin (sometimes called "**slime**"), that facilitates adherence to bioprosthetic material surfaces, such as intravenous catheters, and acts as a barrier to antimicrobial agents.

Staphylococcus saprophyticus

- It normally inhabits the skin and genital mucosa.
- The bacterium causes: - Urinary tract infection (Cystitis) by endogenous spread in colonized women and the symptoms are (dysuria, pyuria, and hematuria).
- Urethritis, catheter-associated urinary tract infections and prostatitis in elderly men. Urinary tract infection caused by S**. *saprophyticus*** can be treated with quinolones (such as norfloxacin) or with trimethoprim–sulfamethoxazole.

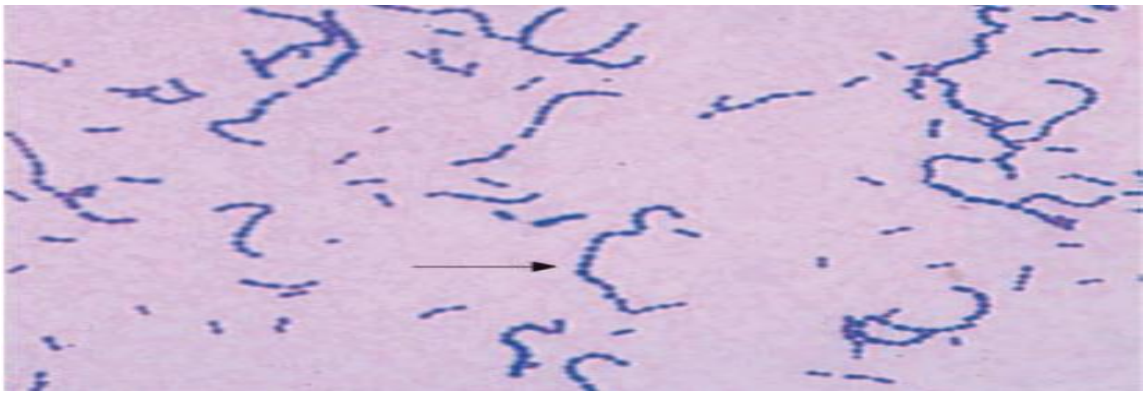
Species	Frequency of disease	Coagulase	Color of colonies	Mannitol fermentation	Novobiocin resistance
S. aureus	Common	+	Golden yellow	+	–
S. epidermidis	Common	–	White	–	–
S. saprophyticus	Occasional	–	Variable	–	+

Summary of various species of staphylococci.

Lecture 6:

Streptococcus

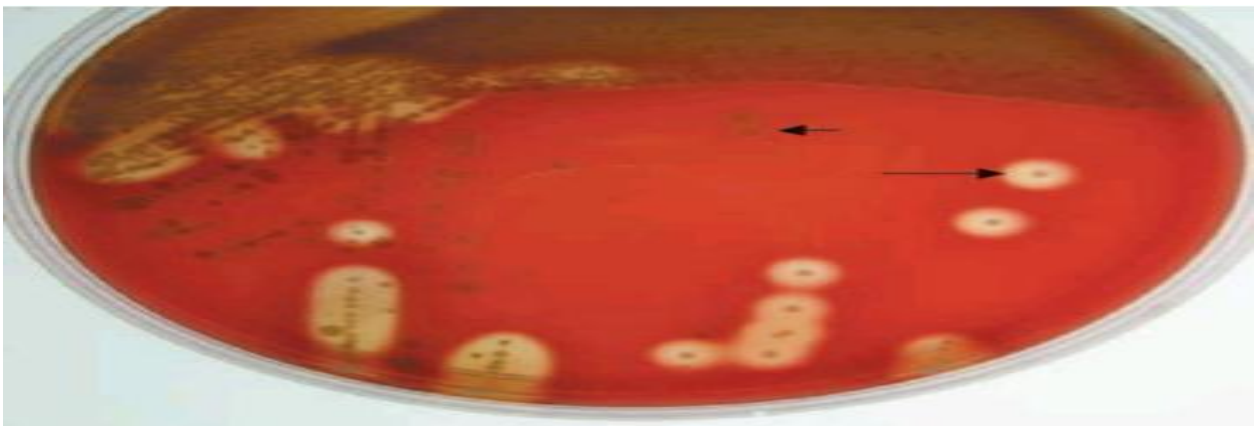
- Streptococci are **spherical** gram-positive cocci arranged in chains or pairs All streptococci are catalase negative, whereas staphylococci are catalase-positive, non- motile , non-spore forming ,and aerobic or facultative an aerobic
- M protein is the most important virulence factor of *St.Pyogenes* which consider anti-phagocytic.



***Streptococcus pyogenes*—Gram stain. Arrow points to a long chain of gram-positive cocci**

Classification system is based on hemolysis reactions:

- α-Hemolytic streptococci form a green zone around their colonies as a result of incomplete lysis of red blood cells in the agar, e.g. *Streptococcus pneumonia*.
- β-Hemolytic streptococci form a clear zone around their colonies because complete lysis of the red cells occurs. β -Hemolysis is due to the production of enzymes (hemolysins) called streptolysin O, e.g. Streptococcus pyogenes.
- γ-hemolysis are non-hemolytic, *Enterococcus faecalis*.



α-Hemolysis and β-hemolysis on blood agar— Short arrow points to an α-hemolytic colony, a viridans group Streptococcus. Long arrow points to a β-hemolytic colony, *Streptococcus pyogenes*.

Streptococcus pyogenes

It is the most important human pathogen causing:

1. Pyogenic infections, such as bacterial pharyngitis and cellulitis.

2. Toxin-mediated diseases, such as **scarlet fever** and toxic shock syndrome.

3. Immunologic diseases, such as acute glomerulonephritis and rheumatic fever.

Pathogenesis:

- **Transmission** through contact; droplets, food and fomites.
- Portal of entry is generally through skin or pharynx.
- Children are predominant group affected for cutaneous and throat infections.
- **M-protein**: antiphagocytic, associated with acute glomerulonephritis, rheumatic fever
- **Streptolysin O**: immunogenic, hemolysin/cytolysin .

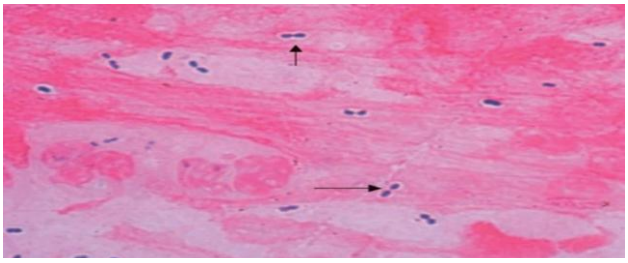
Diseases:

- Acute pharyngitis or pharyngo tonsillitis
- Acute rheumatic fever
- Puerperal sepsis
- Invasive group A streptococcal disease

Treatment:

- Penicillin G
- Erythromycin who are allergic to penicillin.

Streptococcus pneumonia



- α- hemolytic
- **Lancet-shaped diplococci**
- **Contain a capsule**.
- It will not cause any illness itself unless a viral infection or other factors provokes.
- **Reservoir** : human upper respiratory tract
- **Transmission:** respiratory droplets **(**often colonizes the nasopharynx without causing disease)

Pathogenesis:

- Polysaccharide capsule is the major virulence factor
- IgA protease
- Teichoic acid
- Pneumolysin O: hemolysin/cytolysin: damages respiratory epithelium; inhibits leukocyte respiratory burst

Diseases:

- Pneumonia and otitis media.

Treatment

- Penicillin.

Viridans Streptococci (*St.mutans*)

- gram-positive, catalase-negative
- α hemolytic
- normal flora of human oropharynx
- **Reservoir**: human oropharynx (normal flora)
- Transmission: endogenous

Pathogenesis:

- biofilm-mediated adherence onto tooth **enamel**(dental caries) or damaged heart valve and to each other (vegetation); growth in vegetation protects organism from immune system

Diseases :

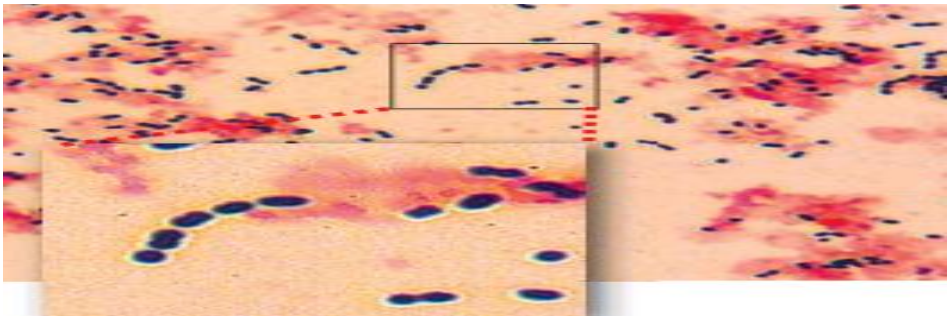
- dental caries
- endocarditis : during a bacteremia

Treatment: penicillin G

Enterococcus faecalis

- Gram (+), cocci, chain formation, catalase (−), variable hemolysis.
- *E. faecalis* normally lives harmlessly in the intestine. However, if it spreads to other parts of the body it can cause a more serious infection. The bacteria can get into your blood, urine, or a wound during surgery. From there, it can spread to different sites causing more serious infections.

- *E. faecalis* bacteria don't usually cause problems in healthy people. But people with underlying health conditions or a weakened immune system are more likely to get sick. These infections often spread in hospitals.



***Enterococcus fecalis* showing chain formation characteristic of Streptococcus**

Pathogenesis:

- Bile/salt tolerance allows survival in bowel and gall bladder.

Diseases:

- Bacteremia, endocarditis, meningitis, UTI and wound infections.

Treatment: Ampicillin.

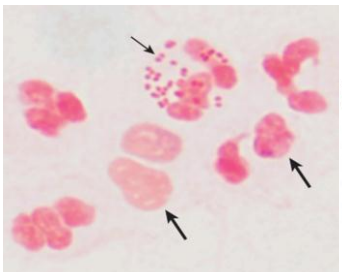
Lecture 7

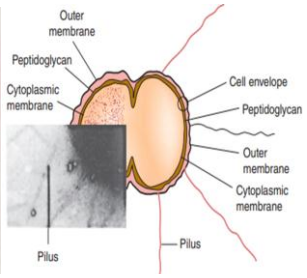
GRAM-NEGATIVE COCCI

NEISSERIA

Genus Features

- ❖ Gram negative
- ❖ Diplococci with flattened sides
- ❖ Oxidase positive





Organism	Vaccine	Capsule	Portal of Entry	Glucose Fermentation	Maltose Fermentation
N. meningitidis	Yes	Yes	Respiratory	Yes	Yes
N. gonorrhoeae	No	No	Genital	Yes	No

Neisseria meningitidis

- ❖ Gram-negative, kidney bean–shaped diplococci .
- ❖ Large capsule.
- ❖ Grows on chocolate (not blood) agar in 5% CO2 atmosphere
- ❖ Ferments maltose in contrast to gonococci
- ❖ **Reservoir**—human nasopharynx (5–10% carriers)
- ❖ **Transmission** :
  - – respiratory droplets; oropharyngeal colonization, spreads to the meninges via the bloodstream
  - – Disease occurs in only small percentage of colonized individuals.

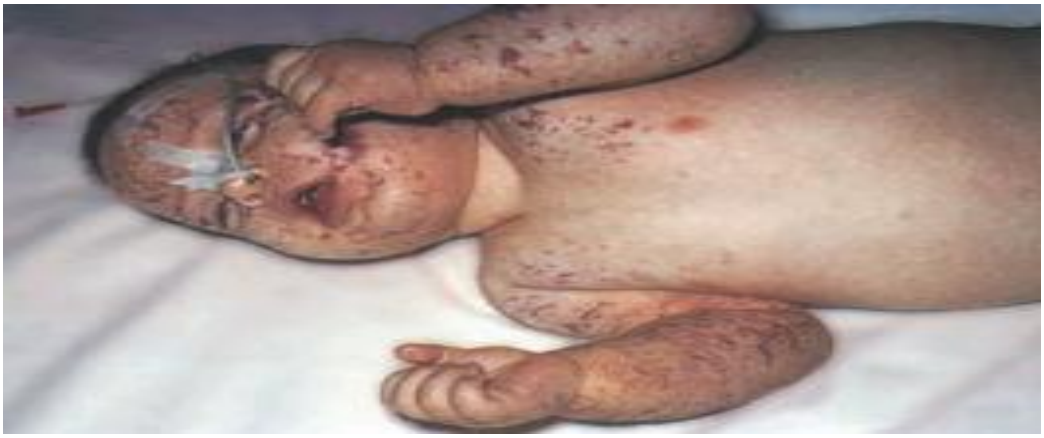
Pathogenesis :

Important virulence factors

- **Polysaccharide capsule**: antiphagocytic, antigenic, 5 common serogroups
- **IgA protease** allows oropharynx colonization.
- **Endotoxin** (lipooligosaccharide): fever, septic shock in meningococcemia, overproduction of outer membrane

Disease(s):

- ❖ Meningitis and meningococcemia – Abrupt onset with fever, chills, malaise, prostration, and a rash that is generally petechial; rapid decline



Petechial and/or purpuric rash and neck extension characteristic of meningococcal meningitis.

Treatment :

- Neonates/infants: ampicillin and cefotaxime
- Older infants, children, and adults: cefotaxime or ceftriaxone with or without vancomycin

Neisseria gonorrhoeae

- Gram-negative, kidney bean–shaped diplococci
- **Reservoir**—human genital tract
- **Transmission** by Sexual contact, birth
- Sensitive to drying and cold

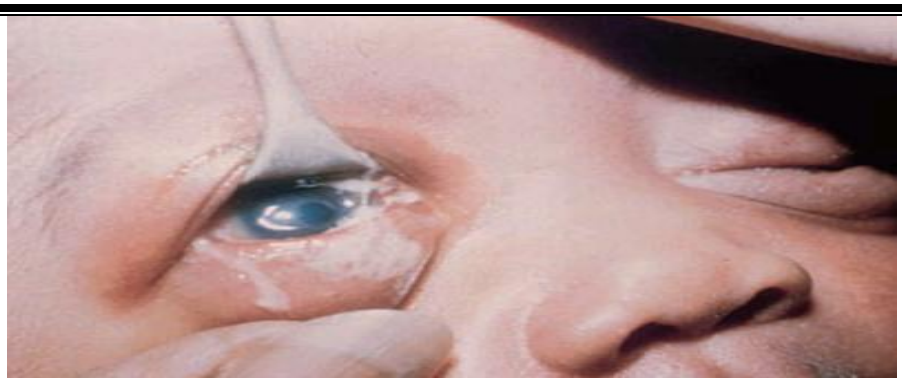
Pathogenesis:

- Pili
  - Attachment to mucosal surfaces
  - Inhibit phagocytic uptake
  - Antigenic (immunogenic) variation
- IgA protease: aids in colonization and cellular uptake
- Endotoxin (lipooligosaccharide)

Organism invades mucosal surfaces and causes inflammation.

Disease : (**gonorrhea)**

- Male: urethritis, proctitis
- Female: endocervicitis, pelvic inflammatory diseases (PID) (contiguous spread), arthritis.
- Infants: ophthalmia (rapidly leads to blindness if untreated)



Neonatal conjunctivitis (ophthalmia) caused by *Neisseria gonorrhoeae*

Treatment

Ceftriaxone

Mycobacterium

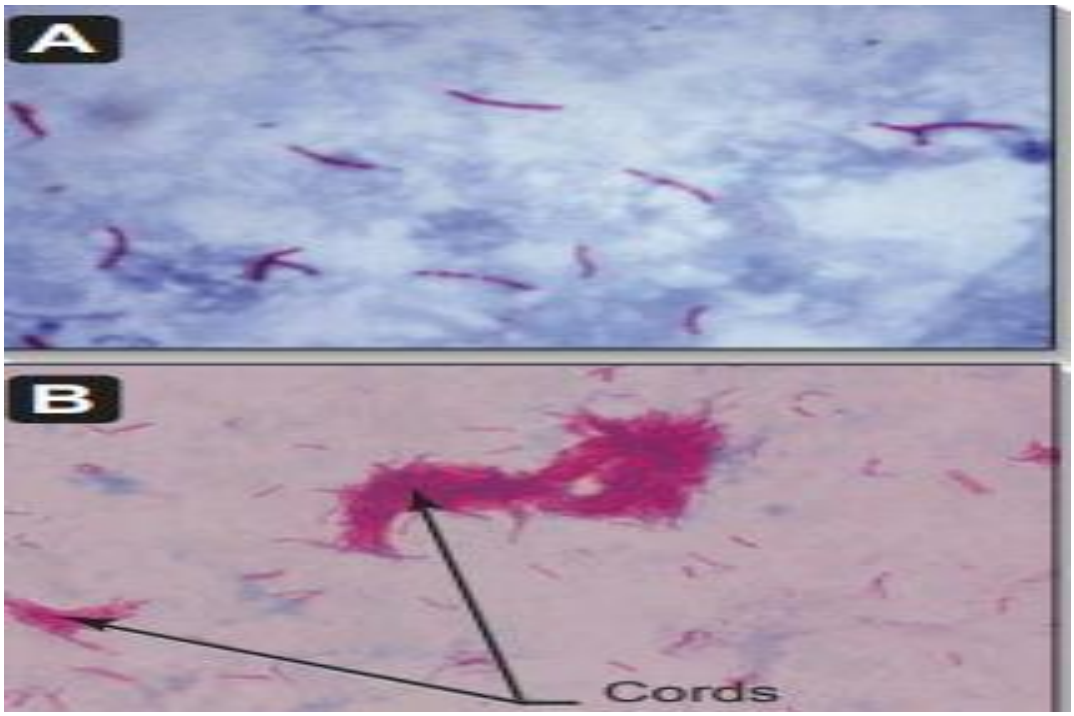
- ❖ Acid fast rods with a waxy cell wall
- ❖ Obligate aerobe
- ❖ Cell wall
  - – Unique: high concentration of lipids containing long chain fatty acids called **mycolic acids**
  - – Wall makes mycobacteria highly resistant to:
    - ➢ Dehydration
    - ➢ Many chemicals ex : NaOH., and H_2SO_4

Species of Medical Importance

- ❖ ***M. tuberculosis***
- ❖ ***M. leprae***

Mycobacterium tuberculosis

- ➢ Acid fast
- ➢ Aerobic, slow growing on **Lowenstein-Jensen medium**.
- ➢ Produces niacin
- ➢ **Reservoir**: (human lungs)
- ➢ **Transmission**: (respiratory droplets)



***Mycobacterium tuberculosis*. A. Acid-fast stain of sputum from a patient with tuberculosis. B. Typical growth pattern showing "cord factor"**

Pathogenesis :

- **Facultative intracellular organism** (most important)
- **Sulfolipids** in cell envelope
  - Inhibit phagosome-lysosome fusion, allowing intracellular survival (if fusion occurs, waxy nature of cell envelope reduces killing effect)
- **Cord factor**
  - Inhibits leukocyte migration; disrupts mitochondrial respiration and oxidative phosphorylation

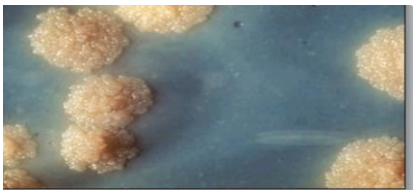
- **Tuberculin** (surface protein) along with **mycolic acid** → delayed hypersensitivity and cell-mediated immunity (CMI)
  - Granulomas and caseation mediated by CMI
  - No exotoxins or endotoxin; damage done by immune system

Disease

- **Primary pulmonary tuberculosis:**
  - most people heal without disease
- **Reactivational tuberculosis:**
  - Complex disease with the potential of infecting any organ system
  - May disseminate (miliary TB)

Identification

- **Microscopy of sputum**: with acid fast stain
- **Skin test (Mantoux test or tubrculin test)**
- Quantiferon-TB Gold Test
- Slow-growing (3–6 weeks) colonies on **Lowenstein-Jensen medium** (faster new systems)
- Produces niacin



Treatment :

Multiple drugs critical to treat (Isoniazid, rifampin, ethambutol, streptomycin, and pyrazinamide)

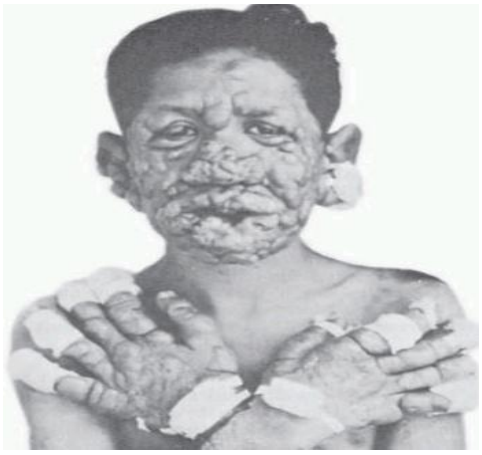
Mycobacterium leprae

- Acid fast rods (seen in punch biopsy)
- Obligate intracellular parasite (cannot be cultured in vitro)
- Optimal growth at less than body temperature
- **Reservoir** :
  - Human mucosa, skin, and nerves are only significant reservoirs
  - Some infected armadillos
- **Transmission**: nasal discharge

Pathogenesis :

- Obligate intracellular parasite
- Cooler parts of body, e.g., skin, mucous membranes, and peripheral nerves

Disease(s): leprosy



Leprosy in a 13-year-old boy in 1931.

Diagnosis:

- Punch biopsy or nasal scrapings; acid fast stain

Treatment:

multiple-drug therapy with **dapsone .**

Lecture8

Clostridia: general characters **:**

- Gram-positive rods
- Spore forming
- Anaerobic

Species of Medical Importance

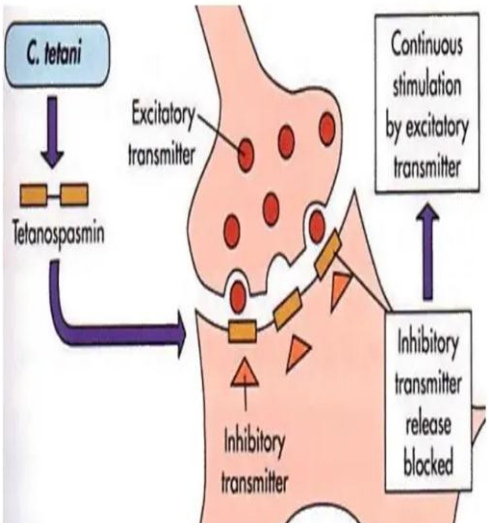
- *Clostridium tetani*
- *Clostridium botulinum*
- *Clostridium perfringens*
- *Clostridium difficile*

Clostridium tetani : Important Features

- ❖ Large gram-positive, spore-forming rods
- ❖ Anaerobes
- ❖ Produces tetanus toxin
- ❖ Reservoir : soil
- ❖ Transmission
  - Puncture wounds/trauma (human bites)
  - Requires low tissue oxygenation

Pathogenesis:

- Spores germinate in the tissues, producing tetanus toxin (an exotoxin also called **tetanospasmin**).
- Carried intra-axonally to CNS
- Binds to **ganglioside receptors**
- Blocks release of inhibitory mediators at spinal synapses
- Extreme Muscle spasm (spastic paralysis)



Treatment of Actual Tetanus

- Hyperimmune human globulin (TIG) to **neutralize** toxin plus metronidazole or penicillin

Prevention

- Toxoid is formaldehyde-inactivated toxin.
- Care of wounds: proper wound cleansing and care plus treatment

factors enhance the growth of *C .tetani* in the wounds

- Lack of oxygen (anaerobic environment
- Necrotic tissue
- Contamination with Spore

Lecture:9

Clostridium perfringens **(clostridial myonecrosis)**

Important Features:

- Large gram-positive, spore-forming rods (spores rare in tissue), non-motile
- Anaerobic: "stormy fermentation" in milk media
- Double zone of hemolysis
- **Reservoir**: soil and human colon
- **Transmission**: foodborne and traumatic implantation


Clostridium perfringens (on blood agar)

Pathogenesis

- Spores germinate under anaerobic conditions in tissue.
- Vegetative cells produce:
  - **Alpha toxin** is a **lecithinase**. It disrupts membranes, damaging RBCs, platelets, WBCs, endothelial cells → massive hemolysis, tissue destruction, hepatic toxicity.
- **Enterotoxin** produced in intestines in food poisoning: disrupts ion transport → watery diarrhea.

Identification : by **Nagler reaction** (**lecithinase**): egg yolk agar plate

Disease(s) :

- **Gas gangrene (myonecrosis)**
  - Contamination of wound with soil or feces
  - Acute and increasing pain at wound site – Tense tissue (edema, gas) and exudate


Myonecrosis

- Food poisoning
  - Reheated meat dishes
  - Enterotoxin production in gut watery

Treatment

- Gangrene: - clindamycin and penicillin
- Food poisoning: - Self-limiting

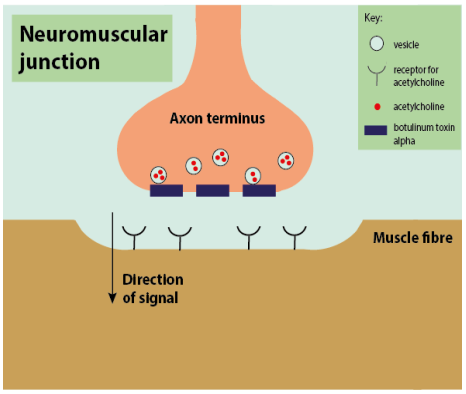
Clostridium botulinum

Important features :

- Anaerobic
- Gram-positive spore-forming rods
- Reservoir: soil/dust
- Transmission: foodborne/traumatic implantation

Pathogenesis

- Spores survive in soil and dust; germinate in moist, warm, and anaerobic conditions
- **Botulinum toxin** − A-B polypeptide neurotoxin
- Highly toxic − Heat labile (unlike staph), 10 minutes 60.0°C
- Absorbed by gut and carried by blood to peripheral nerve synapses Blocks release of acetylcholine at the **myoneuronal junction** resulting in a reversible **flaccid paralysis**



Mechanism action of *c.potulinum* toxin

Symptoms :

- Double vision
- Blurred vision
- Dropping eyelids
- Nausea, vomiting, and abdominal cramps
- Dry mouth
- Muscle weakness
- Constipation

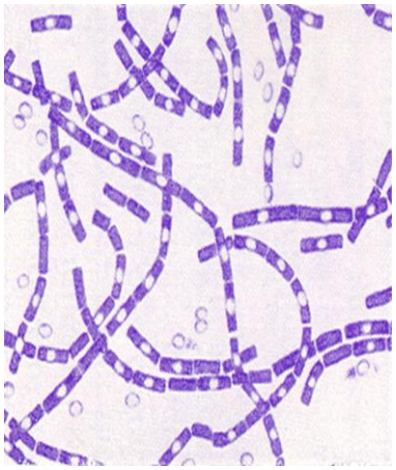
Treatment:

Patients should be hospitalized immediately and treated immediately with **antitoxin** therapy in order to reduce mortality. Immediate intubation is also highly recommended as respiratory failure is the primary cause of death from botulism**.**

Bacillus anthracis

Important features

- ❖ Large, gram-positive, spore-forming rods
- ❖ Capsule is polypeptide
- ❖ Potential biowarfare agent
- ❖ **Reservoir** : animals, skins, soils
- ❖ **Transmission**: contact with infected animals or inhalation of spores (bioterrorism)



Gram stain of *Bacillus anthracis*

Pathogenesis :

- – Capsule: polypeptide, antiphagocytic, immunogenic
- – Anthrax toxin includes 3 protein components.

Diseases:

- ➢ Cutaneous anthrax
- ➢ Respiratory anthrax
- ➢ Gastrointestinal anthrax

Treatment:

Ciprofloxacin or doxycycline

Lecture 10

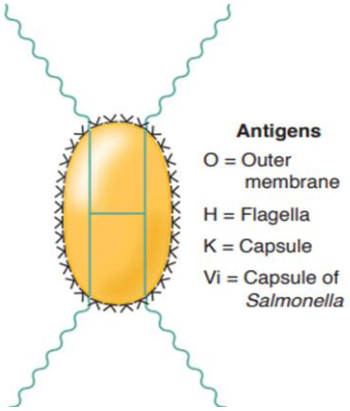
Intestinal bacteria types (Family : Enterobacteriacae)

Important Features

- Gram-negative rods
- Facultative anaerobes
- Ferment glucose
- Oxidase negative
- Reduce nitrates to nitrites
- Catalase positive

Pathogenesis:

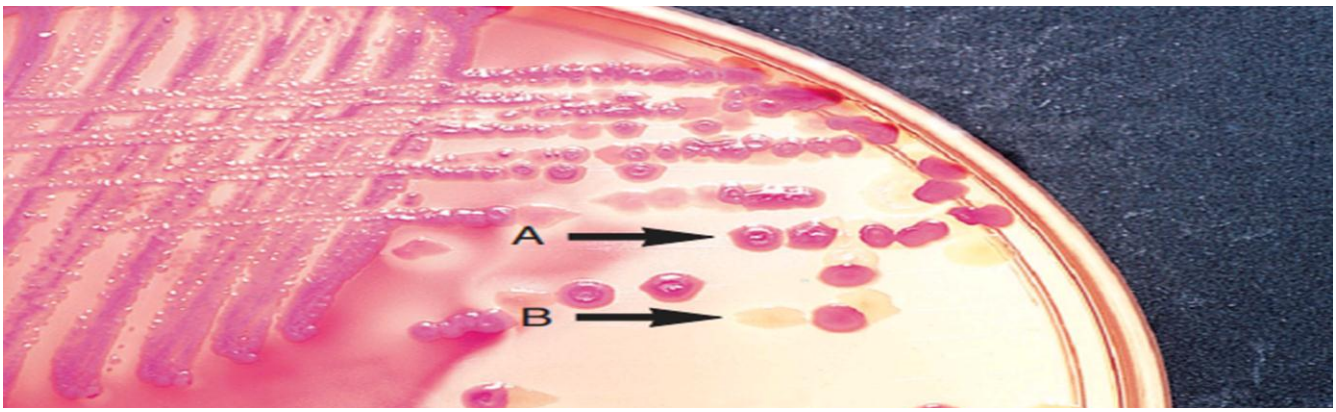
- Endotoxin
- Some also produce exotoxins.
- Antigens
  - **O** = lipopolysaccharide antigen or O Ag
  - H= flagellar (motile cells only) antigen
  - K = capsular polysaccharide antigen
  - Vi (virulence) = *Salmonella* capsular antige



Antigens of Enterobacteriacae

Lab Diagnosis

- Blood agar
- MacConkey agar (differentiate lactose fermentation)
- **Lactose fermenters** (pink color colonies)
- **Non–lactose fermenters** (yellow or colorless colonies)
  - **Lactose Fermenters:** *Enterobacter*, *Escherichia*,and *Klebsiell*
  - **Non−Lactose Fermenters** : Mnemonic: *Shigella* , *Proteus* ,*Yersina* , and *Salmonella*



MacConkey agar as gram negative bacilli : lactose fermenters appear deep purple or pink (A), whereas non lactose fermenters appear yellow or colorless (B).

Salmonella

Medical Importance

- ***S. typhi***
- ***S. enteritidis***
- ***S. typhimurium***
- ***S. paratyphi***
-

Salmonella typhi

Important features

- ❖ Gram-negative rods, highly motile with the Vi capsule
- ❖ Facultative anaerobe, non–lactose fermenting
- ❖ Produces H2S
- ❖ Species identification with biochemical reactions
- ❖ **Reservoir**
  - – **Humans only; no animal reservoirs**
- ❖ **Transmission**
  - – Fecal-oral route from human carriers (gall bladder)

Pathogenesis and Disease

- ➢ Typhoid fever (enteric fever), ***S. typhi*** . milder form: paratyphoid fever; S. paratyphi
  - – Organism ingested (requires large number if stomach acid is normal)
  - – Infection begins in ileocecal region; constipation common
  - – At 1 week: patients have 80% positive blood cultures; 25% have rose spots (trunk/abdomen)
  - – Liver and spleen are infected with additional release of bacteria to bloodstream → signs of septicemia (mainly fever).
  - – Vi capsular antigen (*S. typhi* only) withstands complement-mediated killing.
  - – Biliary system (liver, gallbladder) is infected, organisms enter intestinal tract in bile.

Symptoms:

- ❖ fever, headache, abdominal pain, constipation more common than diarrhea
- ❖ Complications if untreated: necrosis with perforation (local endotoxin triggered damage), cholecystitis, pneumonia, abscess formation.

Treatment: fluoroquinolones or third-generation cephalosporins

Lecture 11

Shigella

Important features :

- ❖ Gram-negative rod
- ❖ Enterobacteriaceae
- ❖ Non–lactose fermenters (colorless colonies on EMB or yellow on MacConkey)
- ❖ Nonmotile
- ❖ Reservoir
  - human colon only (no animal carriers)
- ❖ Transmission
  - fecal-oral spread, person to person

Disease(s):

- ➢ produces endotoxin ,and shiga toxins
- ➢ Enterocolitis/shigellosis (most severe form is dysentery)
- ➢ producing bloody diarrhea.
- ➢ Fever ; lower abdominal cramps; tenesmus; diarrhea first watery, then bloody.

Treatment

- Mild cases: fluid and electrolyte replacement only
- Severe cases: antibiotics

Proteus :

Important features:

- ➢ Gram-negative rod
- ➢ Enterobacteriaceae
- ➢ Peritrichous flagella/highly motile/"**swarming** motility"
- ➢ Non–lactose-fermenting
- ➢ Urease positive
- ➢ **Reservoir**
  - human colon and environment (water and soil)
- ➢ **Transmission**
  - Endogenous

Pathogenesis:

- ➢ Urease raises **urine pH** to cause **kidney stones** (renal calculi).
- ➢ **Motility** may aid entry into bladder.
- ➢ **Endotoxin** causes fever and shock when septicemia occurs.

Disease(s)

- ➢ Urinary tract infections
- ➢ Septicemia

Diagnosis

culture of urine for **lactose-negative organisms** with **swarming** motility.

Escherichia coli

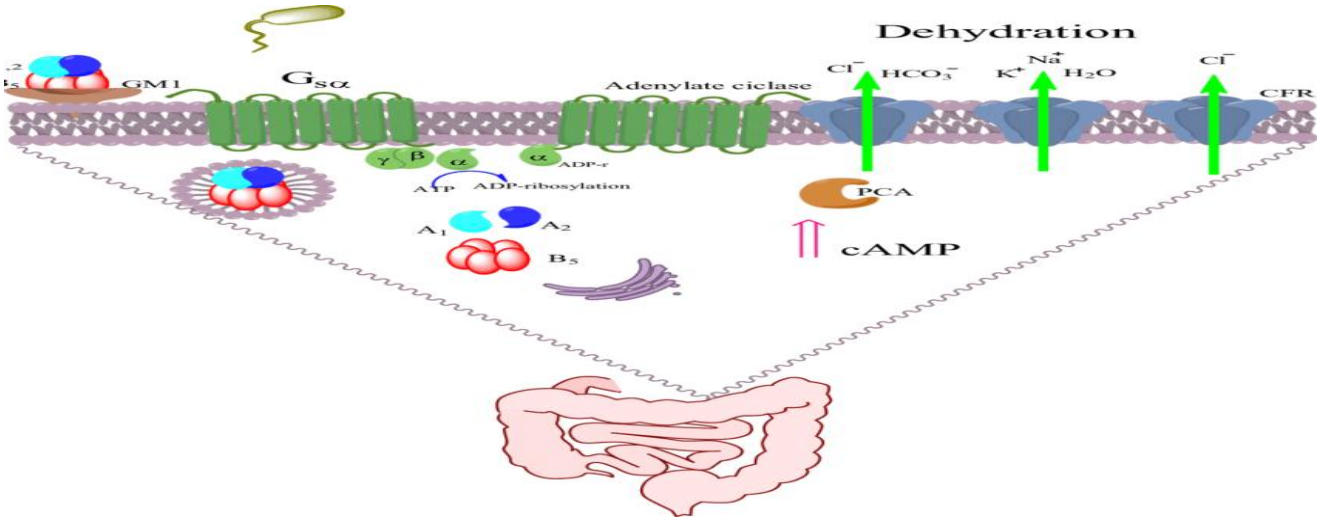
- harmless commensal and also as a versatile pathogen. E. coli in humans causes a broad spectrum of diseases.
- Gram-negative, bacillus
- Enterobacteriacae
- Motile
- facultative anaerobe.

Virulence factors :-

- **Pili** (Fimbriae): play an important role in pathogenesis of urinary tract infection (UTI) caused by *E. coli*.
- **Endotoxin**: Endotoxin is responsible for many of the systemic manifestations of Gram negative bacteria caused by *E. coli* infections. Endotoxin also protects the bacillus from phagocytosis and from the bactericidal effects of complement.
- **Capsule**: protect *E. coli* from phagocytosis.

Pathogenesis:

- **UTIs** , are endogenous are caused by the E. coli present in large numbers in the gastrointestinal tract of the same host. Characterized by **motility** of bacteria and **adherence** to uroepithelium by **pili** .
- **Neonatal meningitis**, are caused by exogenous infections, i.e., acquired from outside ,**K1 capsular antigen, and endotoxin** are associated with this infection .
- Gastroenteritis : by
  - **exotoxins (enterotoxin)**
    - ST (heat stabile toxin)
    - Verotoxin (*Shigella*-like toxins)
    - LT (heat-labile toxin) ; ADP ribosylates (Gs alpha) activating adenylate cyclase → increased cAMP → efflux of Cl– and H2O (persistent activation of adenylate cyclase)
  - **Capsule**



Action of enterotoxin (L.T) *E.coli* as the same as *vibrio cholera* toxin

Diseases:

- Urinary tract infections
- Gastroenteritis
- Septicemia
- Neonatal meningitis.

Treatment *E. coli* usually sensitive to commonly used antibiotics .

Lecture 12

Brucella:

Medical importance ***Brucella abortus***: (cattle)

Important features

- Gram-negative rods
- aerobic
- Zoonosis
- Facultative intracellular pathogen
- Potential biowarfare agent
- **Reservoir**
  - ❖ domestic livestock
- **Transmission**
  - – Unpasteurized dairy products
  - – Direct contact with the animal, work in slaughterhouse

Disease

- **Brucellosis** (**undulating fever**)
- **Acute septicemias** – Fever 37.7–40 °C (often in evening)
- **Influenza-like symptoms**, including arthralgia, anorexia, myalgia, back pain – Sweating (profuse)
- **Hepatomegaly**

Diagnosis

- Culture is hazardous.
- Serum agglutination test.(**Rose Bengal test**)

Treatment

- Adults: rifampin and doxycycline minimum of 6 weeks
- Children: rifampin and cotrimoxazole

Bordetella

Medical importance: ***Bordetella pertussis***

Important features

- Gram-negative small rods
- Strict aerobes Species
- Encapsulated organism
- **Reservoir**: human (vaccinated)
- **Transmission**: respiratory droplets

Disease:

- Whooping Cough **(Pertussis): three stages:-**
  - **Catarrhal**
  - **Paroxysmal**
  - **Convalescent**

Diagnosis:

- **Regan-Lowe or Bordet-Gengou media**.
- Immunofluorescence (DFA) on nasopharyngeal smear
- PCR and serologic tests available

Treatment

- Supportive care; hospitalization if < 6 months old , rythromycin
- Macrolides can also be given.

Lecture 13

Vibrio

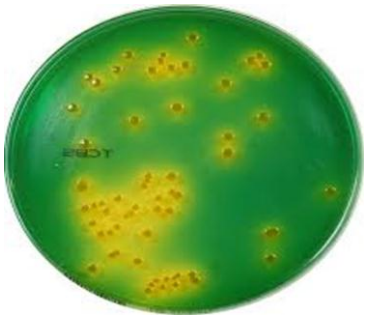
Medical Importance:

- ***Vibrio cholerae***
- ***Vibrio parahaemolyticus***

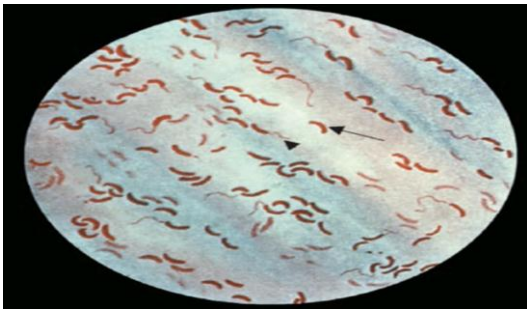
Vibrio cholera

Important features

- Gram-negative curved rod with polar flagella (**Comma shape**)
- Oxidase positive
- Growth on alkaline, but not acidic, media (**TCBS**, thiosulfate citrate bile salt sucrose medium)
- "**Shooting star**"**motility** inactivated by specific serum
- **Reservoir**
  - ❖ Human colon; **no vertebrate animal carriers** (shellfish may be contaminated by water contamination)
- **Transmission**
  - ❖ Fecal-oral spread; sensitive to stomach acid
  - ❖ Requires high dose (>10^7 organisms), if stomach acid is normal



TCBS medium



***Vibrio cholerae*—Gram stain. Long arrow points to a curved gram-negative rod.**

Pathogenesis:

- ➢ Motility, mucinase, and toxin coregulated pili aid in attachment to the intestinal mucosa.
- ➢ Cholera enterotoxin (**choleragen**) similar to ***E. coli* LT**; ADP ribosylates (Gs alpha) activating adenylate cyclase → increased cAMP → efflux of Cl– and H2O (persistent activation of adenylate cyclase)

Disease

- Cholera
- Rice water stools, massive fluid loss
- Hypovolemic shock if not treated

Diagnosis

- Culture stool on **TCBS**
- Oxidase positive

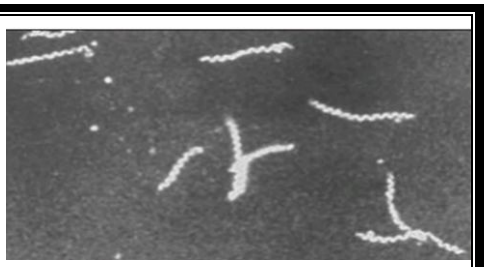
Treatment

- Fluid and electrolyte replacement
- Doxycycline or ciprofloxacin shorten disease and reduce carriage
- Resistance to tetracycline reported

Lecture 14

Tryponema:

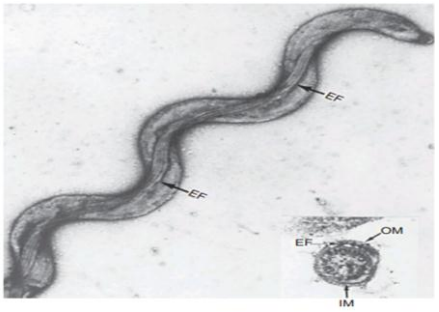
Medical Importance: ***Treponema pallidum***



Dark-field microscopy
Treponema pallidum

Important Features

- Spiral with axial filament (endoflagellum)
- Poorly visible on Gram stain but gram-negative envelope
- Outer membrane has endotoxin-like lipids.
- Axial filaments = endoflagella = periplasmic flagella
- Cannot culture in clinical lab; serodiagnosis
- Is an obligate pathogen (but not intracellular)
- **Reservoir**: human genital tract
- **Transmission**: transmitted sexually or across the placenta



Electron micrograph *T. pallidum* . The endoflagella are clearly visible.

Disease: **Syphilis** (primary, secondary tertiary, and Congenital syphilis)

Diagnosis :

- Visualize organisms by immunofluorescence or **dark-field microscopy**.
- Serology important—two types of antibody:
  - **Nontreponemal antibody** nonspecific (= reagin) screening tests
    - Ab binds to cardiolipin
      - An antigen found in mammalian mitochondrial membranes and in treponemes
      - Cheap source, which is used in screening tests (VDRL, RPR).
      - Very sensitive in primary º
      - But not specific; confirm with FTA-ABS
    - Examples:
      - Venereal disease research lab (VDRL)
      - Rapid plasma reagin (RPR)
  - **Specific tests for treponemal antibody** (more expensive)
    - Fluorescent treponemal antibody-absorption (FTA-ABS; most widely used test)
    - *Treponema pallidum* microhemagglutination (MHA-TP)

Treatment:

- Benzathine penicillin for primary and secondary syphilis (no resistance to penicillin); penicillin G for congenital and late syphilis

lebtospira **:-**

Medical Importance: ***Leptospira interrogans***

Important Features :

- Spiral thin, with tight terminal hooks
- Too thin to visualize, but gram-negative cell envelope
- Seen on dark-field microscopy but not light microscopy
- Can be cultured in vitro; aerobic
- Generally diagnosed by serology
- **Reservoir**: wild and domestic animals (**zoonosis**)
- **Transmission**
  - Contact with animal urine in water. Organism penetrates mucous membranes or enters small breaks in epidermis

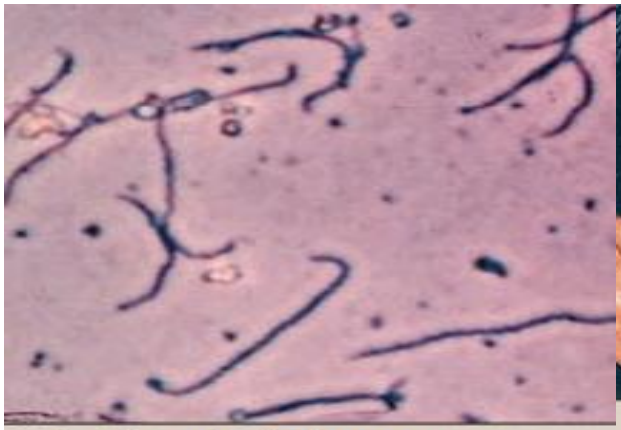
Disease:

- **Leptospirosis** (swamp or mud fever)
- Influenza-like disease ± gastrointestinal tract symptoms
- Progressing on to **hepatitis** and **renal failure** if not treated

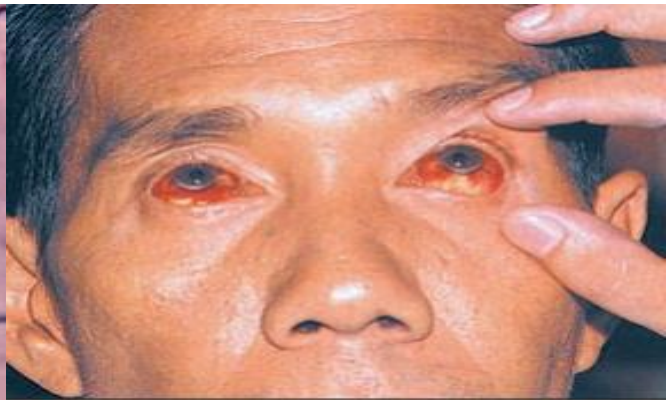
Diagnosis:

- Serodiagnosis (agglutination test)
- Culture (blood, CSF, urine) available in few labs
- Dark-field microscopy insensitive

Treatment: penicillin G or doxycycline



Leptospira interrogans



Scleral hemorrhages in a jaundiced patient with leptospirosis

Lecture 15

Immunity:

Immunity(immunitas-a Latin word ; **freedom from disease**), the way in which the body can protect itself from invasion by pathogenic microorganism and provide a defense against their harmful effect.

General function of immune system

1. Defense against foreign invaders.
2. Auto tolerance (unresponsiveness to the self-tissues)
3. Surveillance: recognition and clearance of internal antigens (old, damaged, or mutagenic cells,…).

The body's defense mechanisms can be divided into: **innate immunity (**non-specific or natural immunity) defense and **adaptive immunity(**specific or acquired immunity).

Innate immunity: is an **immediate**, **nonspecific** response to a pathogen and is the resistance that an individual possesses by birth. The innate immunity is primarily dependent on four types of defensive barriers:

a. anatomic barriers
b. physiologic barriers
c. phagocytosis
d. inflammatory responses.

A- Anatomic barriers:

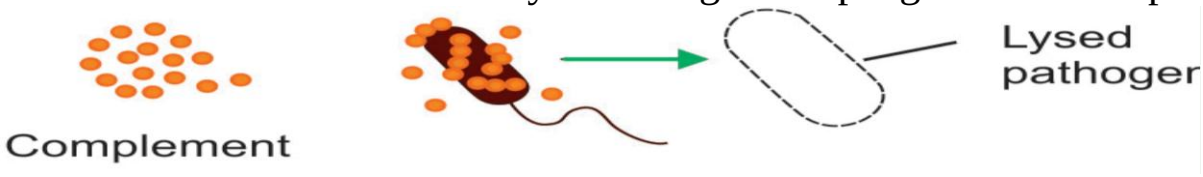
- **Skin**: Mechanically prevents entry of microbes Sweat contains fatty acids which denatures microbial cellular proteins .
- **Mucus**: is a viscous fluid secreted by the epithelial cells of mucous membranes that entraps invading microorganisms.
- **Cilia**: cilia propulsion on epithelia cleans lungs of invading microorganisms.

B- Physiological barriers:

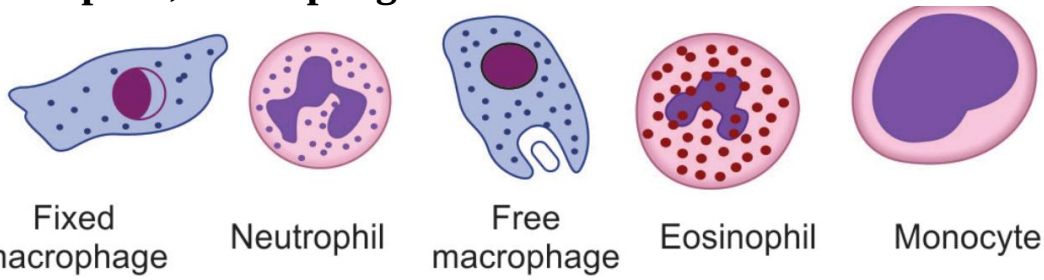
- **Gastric acidity** is an innate physiologic barrier to infection because very few ingested microorganisms can survive the low pH of stomach contents.
- **Lysozyme** has antibacterial effect due to its action on the bacterial cell wall (**tears** and **saliva**).
- **Interferon's** are secreted by cells in response to products of viral infected cells. These substances have a general antiviral effect by preventing the synthesis of viral structural proteins.

Released by activated lymphocytes and macrophages and by virus-infected cells

- **complement system** :consists of a number of **small proteins**; it does three functions:
  - **Membrane attack** : by rupturing the cell wall of bacteria. •
  - **Opsonization** : is a term used to describe how antibodies can enhance phagocytic engulfment of microbes.
  - **Inflammation** : by attracting macrophages and neutrophils.



C- **Phagocytosis**: is a process of ingestion of extracellular particulate material by certain specialized cells, such as blood **monocytes**, **neutrophils**, **macrophages** and **dendritic cells**.

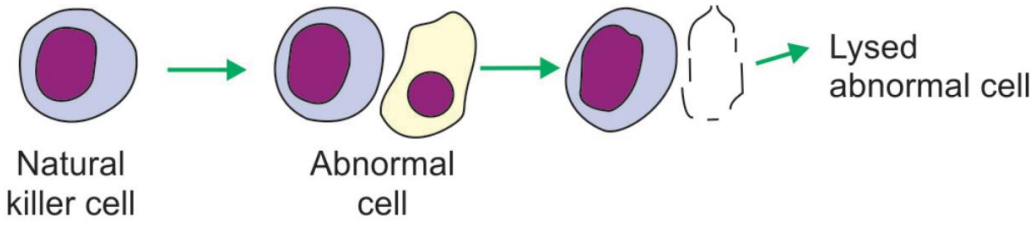


Phagocytes

D- **Inflammatory responses**: tissue damage caused by a wound or by an invading pathogenic microorganism **induces a complex sequence of events**, collectively known as the inflammatory responses. The four cardinal features of inflammatory responses are:

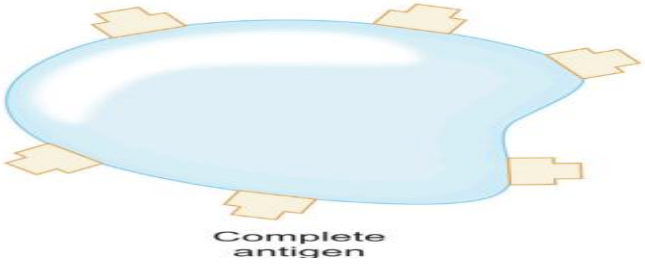
– Redness
– rise in temperature
– pain
– swelling

Natural Killer (NK) cells: Identify and kill virus-infected and tumor cells.

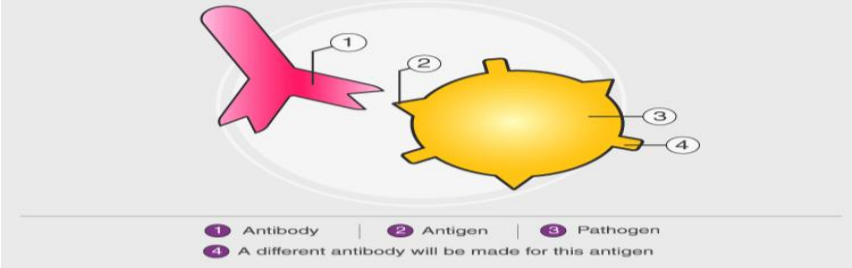


N.K cells

Antigen: a substance capable of inducing a specific immune response.

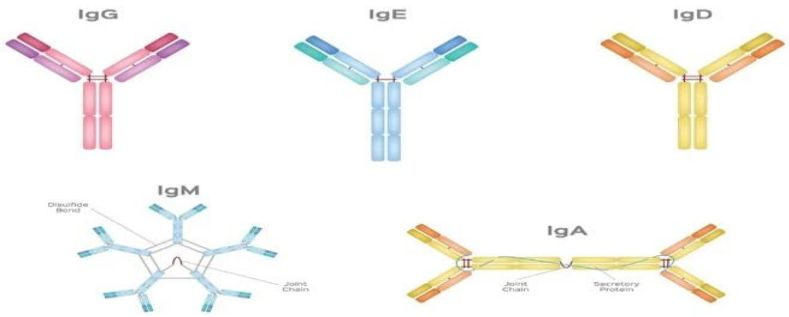


Antibody: a protein (immunoglobulin) that recognizes a particular antigen and binds specifically to it.



Classification of antibodies:

- **IgG** - Crosses placenta, therefore important in protecting newborns - Part of secondary immune response.
- **IgM** - Important in primary immune response.
- **IgA** -Found in body secretions, e.g. mucus membranes.
- **IgE** -Involved in allergic response and the response to helminths.
- **IgD** - Complete function not known.



Types of Antibodies

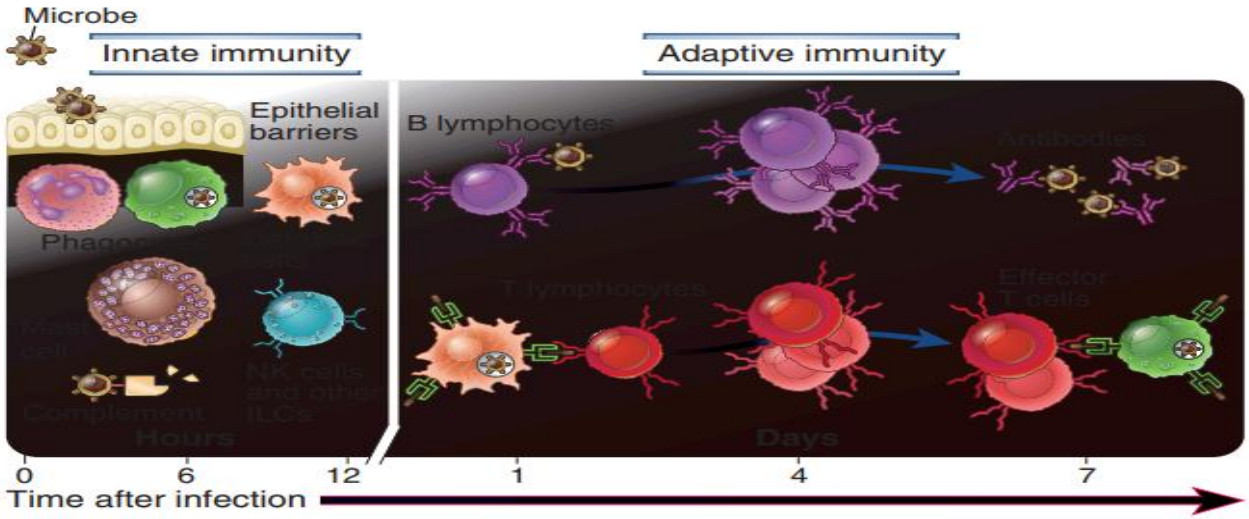
Adaptive immunity: the resistance that an individual **acquires during life**.
Characteristics

- Antigen specific
- Can form memory
- Requires priming- specific cells help to start the acquired immune response

Cells of the acquired immune system:

- **T lymphocytes cells**:
  - **Helper T cells**: recognize antigen, help B cells to make antibodies and T cells to kill.
  - **Cytotoxic T cells**: poisonous to cells, kill cells infected by viruses and intracellular bacteria.
- **B lymphocytes cells**: - Make **antibodies** and **memory cells**.

Cytokines: **Small proteins** that carry **messages** from one cell to another, e.g. to stimulate **activation** or **proliferation** of **lymphocytes** and send **messages** to other **cells**, e.g. to kill or secrete. Cytokines have been classed as **lymphokines**, **interleukins**, and **chemokines**.



Innate and adaptive immunity

Some of the important definitions of microbial diseases and pathogenesis

- **Pathogen :** any microbe that has the potential to cause disease
- **Pathogenesis :** mechanism and stapes by which microbe causes disease
- **Pathogenicity:** ability of an organism to cause disease
- **Virulence:** is the degree of pathogenicity within a group of organisms.
- **Bacteremia:** bacteria present in the blood.
- **Septicemia:** is the presence and multiplication of bacteria in the blood.
- **Opportunistic pathogen :** microbe that typically harmless (normal flora) but can cause disease when host defiance is weakened
- **Infection :** invasion multiplication ,and growth of pathogenic microbes in the tissue of the host
- **Disease:** microbial process with a characteristic set of signs and symptoms