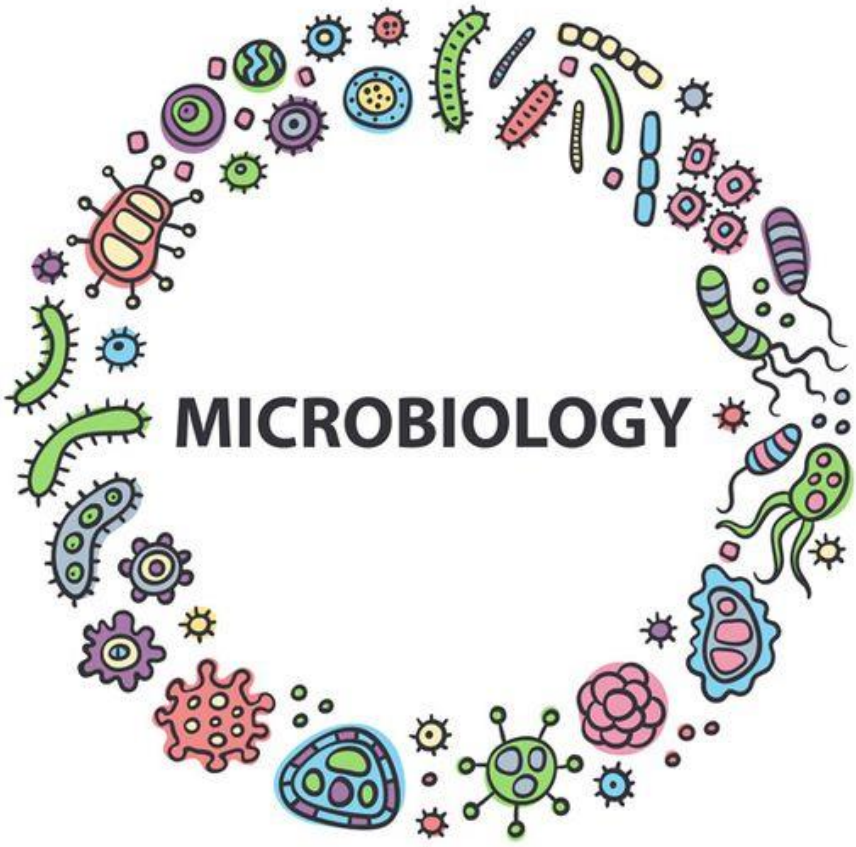
University of Mosul

College of Nursing

Microbiology for Nursing 1



Dr. Mohammed T. Gargary

Assistant Professor

2024-2025

Introduction to Microbiology
The Science of Microbiology

Microbiology and Medicine

Microbiology : is the study of microorganisms, a large and diverse group of microscopic that exist as single cell or cell clusters it also includes viruses, which are microscopic but not cellular.

Medical microbiology :is the study of interactions between humans and the microorganisms, which coexist. Microbiology has also link with curative medicine, diagnosis, treatment of microbial diseases and concerned with an etiology (causation), pathogenesis (mechanism of attack on tissues). Laboratory diagnosis, treatment of infection in the individual and with epidemiology, control or prevention of infection in the community.

Organism Nomenclature

Established by Carolus Linnaeus (1735)

- Latinized

Each organism has unique two parts, Genus and species name:

e.g. (*Escherichia coli*)

- Written in italics or underlined

- Genus with capital first letter

- Species/specific epithet all lowercase

- After first use in documents can abbreviate genus: E. coli

- Name often describes organism: shape, habitat, name of discoverer, etc.

Microorganisms may be grouped according to nature interaction with humans :

1- Normal microbial flora: The term "normal microbial flora" or non-pathogenic routinely colonize body surface without doing harm e.g., *E. coli* in intestinal tract, and *Staphylococcus epidermidis* on skin or on nose.

2- Pathogenic organisms: The pathogenesis of microorganisms infection includes initiation of the infectious process leading to development of signs and symptoms of disease characteristics of pathogens include transmissibility , adherence to host cell, invasion of cell and tissues, toxigenicity, and ability to evade the hosts immune system and damage the human (host) either by direct invasion and injury e.g., *Shigella spp.* cause Shigellosis or by the production of harmful toxic products, e.g. *Clostridium spp.* cause tetanus and Gas gangrene. *Salmonella typhi* cause (Salmonellosis) .

3. Opportunistic organisms: These usually found in the environment or as part of the normal flora, these are in normal individuals are harmless, but they may make or cause severe disease in immunocompromised patients or they penetrate a territory from which they are usually excluded as result. For example of trauma or surgery. e.g. *Staph. epidermidis* on skin , *Neisseria spp*. and Diphtheroids in nasopharynx.

4. Zoonotic organisms: Some organisms are worldwide distribution that infects a wide range of animals and birds usually cause disease in human such as parasitizes ,sporozooans and trematodes in vertebrates other than humans but may by acquired through contact with infected animals or animal products e.g., in malaria disease, by the blood sucking bite of female Anopheles mosquitoes by *Plasmodium malariae* , in toxoplasmosis disease, by *Toxoplasma gondii* and in Leishmaniasis infection by sand flies by *Leshmania donavani* .

Classification of Microorganisms

All living organisms on earth are composed of one or the other of two types of cells: prokaryotic cells and eukaryotic cells based on differences in cellular organization and biochemistry (Table 1.2).

1. Prokaryotes: All bacteria and blue-green algae are prokaryotes.

2. Eukaryotes: Other algae (excluding blue-green algae), fungi, slime molds, Protozoa, higher plants and animals are Eukaryotes.

Table 1.1: distinguishing characteristic of Prokaryotic and Eukaryotic.

Character	Prokaryotic	Eukaryotic
1. Size	1-10 µm	10-100 µm
2. Nucleus	Absent	Present
- Nuclear membrane	Absent	Present
-Nucleolus Deoxyribonucleic protein	Absent	Present
-Chromosome	One	More
-Meiotic division	Absent	Present
3. Cell wall comp.	Peptidoglycan	Chitin, cellulose
4. Steroids	Absent	Present
5. No. of ribosome1	70s	80s
6. Cytoplasm part	Absent	Present
- mitochondria	Absent	Present
- lysozyme	Absent	Present
- Golgi apparatus	Absent	Present
- Endoplasmic reticular		
7. Chemical composition	Present	Absent
- muramic acid	May be present	Absent
- diaminopimelic acid		
8. Nitrogen fixation	Possible	Not possible
9. Respiration	Anaerobic possible	No

Microbial virulence:

Microbial virulence is the ability of microorganism to cause disease. All the factors that contribute to microbial pathogenesis are known as virulence factors. Pathogenic bacteria may be have one or several virulence factors, but scientists can use virulence factors in application medicine as component vaccines based on the use of modified virulence factors that lack toxic effects but retain immunogenicity.

❖ **Types of virulence factors:**

1- Capsules: Capsules located externally to cell wall enable the bacterium to avoid of survive phagocytosis and adherence of bacteria to surface in their environment, including the cell of host. Bacteria capsulated are virulent and harm to the host, the composition of it often polysaccharide. Such as *Streptococcus pneumoniae* , *Shigella, Salmonella* .

2. Adhesions : Many bacteria depend on the ability to adhere to mucosal cell as first step in the infectious process, is followed by development of micro colonies and steps in pathogenesis of infection, to causing the disease, without adhesion factors many pharyngeal, intestinal and urinary tract would be washed away before they could cause disease

a. Fimbriae, b. Pili, c. Lipopolysaccharide (LPS).

3. Invasion enzymes : Many bacteria produce and secrete enzymes these enzyme play an important pathogenic role by variety of mechanisms. For example :

a- Enzymes breakdown collagen and fibrin (i.e., collagenase) penetration of bacteria into tissues.

b- Enzymes breakdown cellular material e.g., Proteases, lecithinases .

c- Enzyme modify inactivate antibiotic. e.g., Beta lactamase that provide multi-resistance to β-lactam antibiotics such as penicillins, cephalosporins, cephamycins, and carbapenems (ertapenem).

4. Toxins :Toxins produced by bacteria are generally classified into two groups, Exotoxins and Endotoxin.

a- Exotoxins: are proteins produced and released from the cell to cause toxicity. e.g., *Clostridium tetani* produce the tetanospasmin.

b. Endotoxin: Is part of the bacterial cell wall. eg. Gram (-ve) bacteria as Lipopolysaccharide (LPS). e.g. types of *E. coli*, *V.cholera,* and *Shigella spp.*

Table1.2: The difference between Exotoxins and Endotoxins

	Exotoxins	Endotoxins
1.	Excreted by living cell in liquid media.	Integral part of cell wall of gram (-v) bacteria released on death.
2.	Produced by both gram (–ve) and gram (+ve) bacteria.	Found only in gram (–ve) bacteria.
3.	Protein (polypeptides) with a molecular weight 10,000 – 900,000	Lipopolysaccharide complexes; lipid A portion probably responsible for toxicity
4.	Unstable–destroyed rapidly by heating at temperatures above 60 C.	Stable without loss of toxicity by heating at temperatures above 60 C.
5.	Highly toxic, fatal to animals in microgram or less.	Moderately toxic, fatal for animals in tens to hundreds micrograms.
6.	Active bacteria	Bacterial lysis
7.	Usually do not produce fever in the host.	Usually produce fever in the host .

Morphological and Shapes of bacteria

The first step taken in the identification of an unknown bacterium is macroscopically examination, for examples:-

a. Spherical (round), cocous. e.g., *Staphylococcus, Streptococcus.*

b. Bacillus (cylindrical) as rod e.g., *Clostridium, Salmonella.*

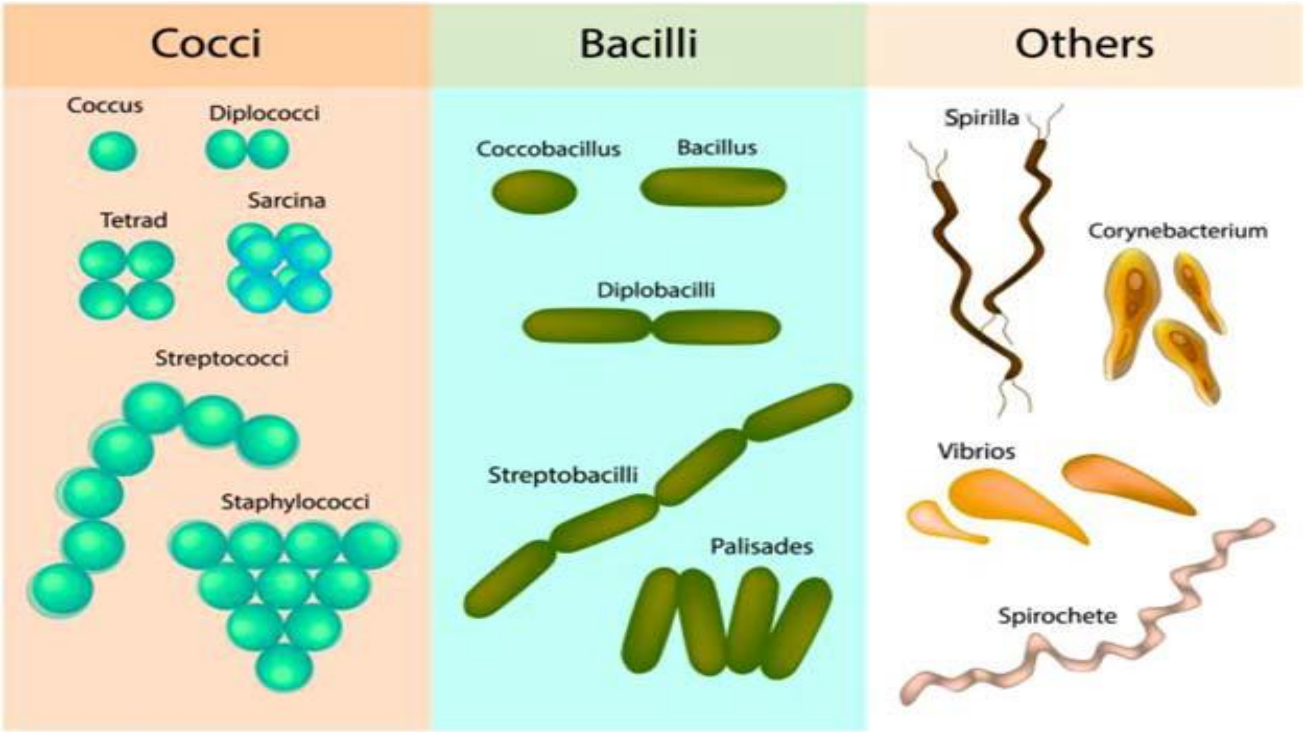
c. Coccobacillus e.g., *Pseudomonas.*

d. Vibrio e.g., *Vibrio cholera , Borrelia spp.*

e. Spirochetes . e.g., *Treponema , Leptospira.*

f. Pleomorphic e.g. *Moraxella spp.*

FIGURE 1.a Shapes of bacteria.



The basic structure of bacteria

Most individual bacterial cells are too small to be seen without a microscope.

All bacteria are prokaryotes:

1- no nucleus , 2- small cells, simple structure

3- usually no organelles , 4- much less efficient design

I- Extracellular structure

1- Cell Wall , 2- Cell membrane (Cytoplasmic or Plasma membrane)

❖ Cell wall

Cell wall thick 10-25 nm, strong relatively rigid and maintain the characteristic shape of the bacteria. It is determine gram-positive or gram-negative depend on the composition of it.

Functions of cell wall:

1- Protects the bacteria.

2- Allows them to live in "extreme" environments.

3- Give it their external shape.

❖ Cytoplasmic membrane

Also terms as plasma membrane is the physical and metabolic barrier between the interior and exterior of the bacterial cell. **The function of cell membrane:**

1- Uptake of nutrients

2- Excretion of waste products

3- Secrets the enzymes

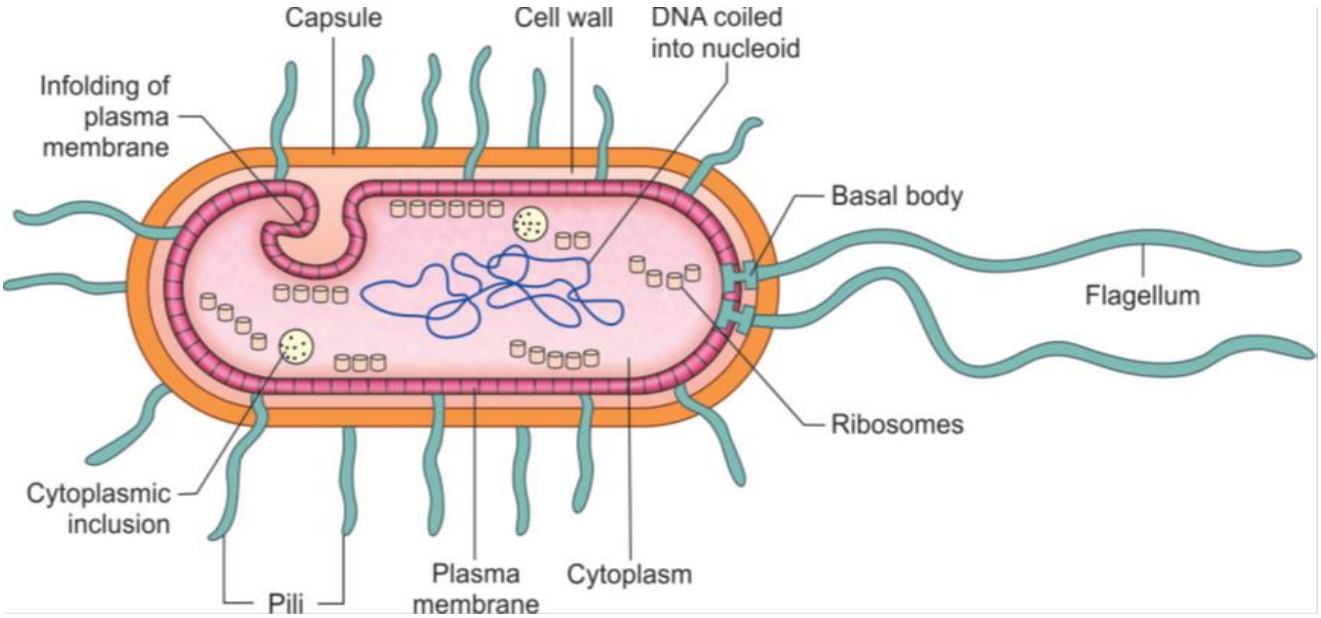


Fig 1.3: The basic structure of bacteria

II- Internal structure of bacteria:

a. DNA: the bacterial cell lacks nuclear membrane in cytoplasm as nucleotide it contain plasmids.

b. Ribosome: are complex globular structures composed of several RNA molecules and proteins they have two sub-units terms 50S and 30S it is important in synthesis of proteins.

c. Volutin granules (source of energy).

III- Bacterial appendages: (Special structures)

a. Capsules: around many bacterial cell are composed of complex Polysaccharides, capsule also terms glycocalyx.

b. Flagella: are present in many bacteria. It responsible for the motility it may be types in :

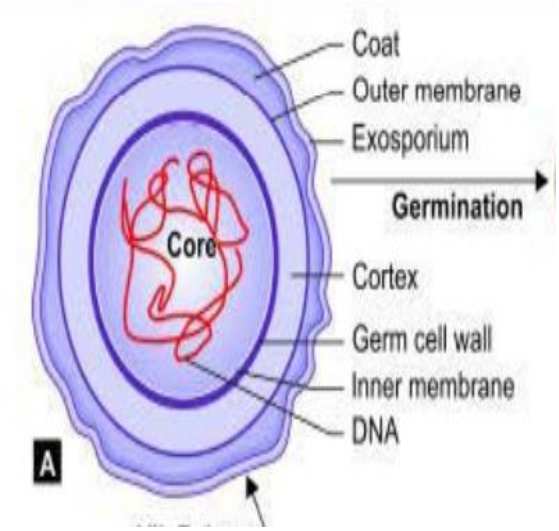
Monotrichous, Lophotrichous, Amphitrichous , Peritrichous.

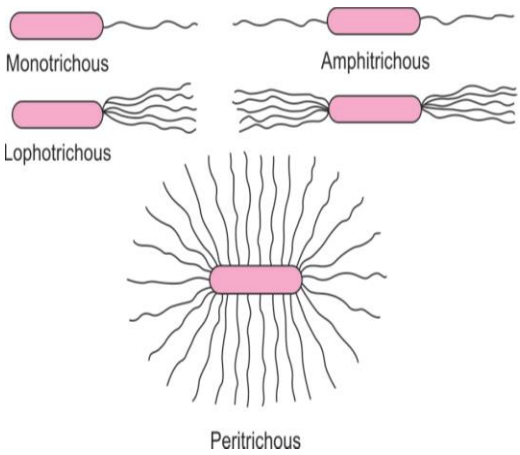
c. Fimbriae :use for adherence as protein fibers cover on the surface cell.

d. Pili: protein structure use for adherence and transport genetic or sex (F) in conjugation , usually coded by plasmids.

e. Endospore (Bacterial spores):

Some species of bacteria are capable of undergo and develop a highly resistant that can survive in a dormant state the along period that resistant to heat, radiation and chemical. In sporulation each vegetative cell forms only one spore and in subsequent germination each spore gives a single vegetative cell. Spore may remain viable for many years.





Bacterial Physiology and Staining Techniques

Microscopic Techniques

Microscope is an optical instrument used to magnify (enlarge) minute objects or microorganisms which cannot be seen by naked eye. Several types of microscopes are used in study of microbiology one of the most important tools for studying microorganisms is the light microscope for observing objects.

Types of microscopes are:

A. Light Microscopy

B. Electron Microscopy

C. Scanning Probe Microscopes

Dyes and Staining

Stain combines chemically with bacterial protoplasm if the cell is not dead the staining process itself kill it. The basic dyes stain bacterial cells. Special staining techniques can be used to differentiate flagella, capsules, cell walls, cell membrane granules, nucleotides and spores.

Types of Dyes and Staining

a- Gram Stain

Bacteria are colorless and are nonvisible under a light microscope. Dyes are used to color them. Some bacteria are mostly gram positive, retain the blue dye (crystal violet), and appear blue under a microscope, while gram-negative bacteria whose outer membrane is dissolved using alcohol do not retain the blue color and take the red color of safranin dye during counterstaining.

Gram Stain divided the bacteria to gram positive and gram negative depend on the cell wall.

Procedure of staining usually involves several steps. First step is fixing the sample on a slide, then use crystal violet dye and wait for 1 minute and then wash off with water, this removes extra dyes from the sample. The sample is flushed with iodine solution and washed off with water and 95% alcohol. This partially dissolves the lipid outer membrane of the gram-negative bacterial cell. Counterstaining with safranin (red dye) makes the gram-negative cell look red (gray in print version) in a light microscope, while gram positive bacteria appear blue or purple (dark gray in print version).

Table 2.1: Steps of gram stain.

Steps in staining	State of bacteria
Step 1: Crystal violet (primary stain)	Cell stain purple
Step 2: Iodine	Cell stain purple
Step 3: Alcohol (decolorizer)	Gram (-ve) cells become colorless
Step 4: Safranin (counter stain)	Gram (+ve) cells remain purple Gram (-ve) cells appear red

Figure 2.1 Difference between gram-positive and gram-negative bacterial cell wall.

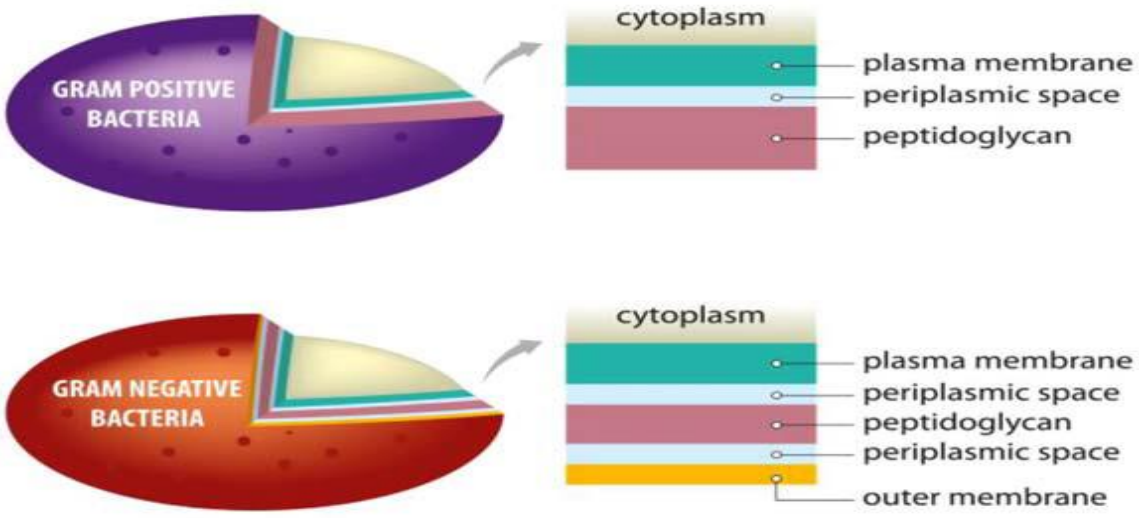


Table 2.2 Difference between gram-positive and gram-negative bacteria.

Gram-positive bacteria(G+)	Gram-negative bacteria(G-)
1-Thick layer peptidoglycan cell wall 2-The outer membrane is absent 3-Teichoic acid is present 4-Porins absent 5-Lipopolysaccharide absent 6-Have exotoxins 7-Stain blue or purple on Gram's staining 8-Periplasmic space absent	1-Thin layer peptidoglycan cell wall 2-The outer membrane is present 3-Teichoic acid is absent 4-Porins present in outer membrane 5-Lipopolysaccharide present 6-Have endotoxin + exotoxins 7-Stain pink or red on Gram's staining 8- Periplasmic space present

b. Acid-fast Stain : Ziehl–Neelsen staining is a type of acid-fast stain, first introduced by Paul Ehrlich. Ziehl–Neelsen staining is a bacteriological stain used to identify acid-fast organisms, mainly Mycobacteria. It is named for two German doctors who modified the stain: the bacteriologist Franz Ziehl (1859–1926) and the pathologist Friedrich Neelsen (1854–1898).

c. Special Stain:

This stain specific structure inside or outside of the cell:

1. Capsule stain. 2. Flagella stain. 3. Endospore stain.

d. Negative Staining:

This staining the background with the acidic dye, leaving the cell colorless.

e. Fluorescent dyes and tags

- **Molecules of bacterial cell:**

All bacterial cells contain a variety of small organic and inorganic molecules, many of which occurred in the form of ions, other are very large molecules .The type of molecules are:

1- Small molecules (Micromolecules):

This as forms of ions, organic molecules contain carbon atoms bonded to each other or to hydrogen atoms, also contain ions principally Na^+, K^{+2}, Mg^{+2}, Ca^{+2}, Fe^+, Cl^-, PO_4^{-3} and SO_4^{-3}.

2. Large molecules (Macromolecules):

Macromolecules are large consisting of several thousand atoms, each four major class of biologically important macromolecules include :

A. Proteins: Proteins constitute more than 50% of the dry weight of cell, proteins are responsible for:

1. Catalyzing all reaction as enzymes.

2. The structure and shape as Ribosomes.

3. Cell movement such as flagella.

4. Taking nutrient into the cell as protein.

5. Turning genes on and off as gene regulations.

6. Structure of membrane as outer and inner.

7. Amino acid as subunit of protein.

B. Nucleic acids

C. polysaccharides (carbohydrate)

D. lipids.

Bacterial Nutritional Requirements

The main elements required for growth are carbon, hydrogen, oxygen and nitrogen with sulfur and phosphorus required in smaller amounts. Other elements such as sodium, potassium, magnesium, iron and manganese also required in smaller amount.

1. Carbon:

a. Autotrophy bacteria use CO2 as sour of carbon.

b. Heterotrophy bacteria require more complex organic compound such as carbohydrates and amino acid.

2. Inorganic ions: Such as PO4, K+, Mg=2, MN, N, S.

3. Organic nutrients: a. Carbohydrate b. Amino acid c. Vitamins

4. Electron donors: Such as ammonia, nitrites, and H2S.

5. Electron acceptors: Such as oxygen, pyruvate, lactate.

6. Oxygen:

Bacteria can be divided into five groups on the basis of their oxygen requirements:

1. Obligate aerobes e.g., *Vibrio cholera* , *Bordetella pertussis.*

2. Obligate anaerobes e.g., *Clostridium tetani , Cl. Welchii* .

3. Facultative anaerobe e.g., *Bacillus anthraces* , Enterobacteriaceae.

4. Microaerophilic bacteria e.g., Campylobacter species , Helicobacter.

5. Aerotolerant bacteria e.g., some of species produce enzymes term superoxide dismutase and peroxidase . use fermentation to produce ATP. An example of an aerotolerant anaerobe is *Cutibacterium acnes* .

Bacteria are divided on the basis of energy sources into:

1. Phototrophic: Which get energy from photochemical reaction.

2. Chemotropic: Which get energy from chemical reaction.

Bacteria are divided on the basis of temperature into:

1. Mesophilic bacteria: Growth best at temp. ranging from 20-40oC. Most human pathogens are Mesophilic at 37oC.

2. Thermophilic bacteria: Growth best at 50-60oC

3. Psychrophilic bacteria: Growth best at temp. ranging from 0o-10oC.

4. Hyperthermophilic bacteria: Can grow a well above the temp of boiling water.

Bacteria are divided on the basis of optimal pH range into:

1. Neutralophilic: Grow best at a pH of (6.0-8.0)Includes most human pathogens.

2. Acidophilic: Grow at very low pH (0.1 to 5.4), Lactobacillus produces lactic acid.

3. Alkaliphilic: Grow at alkaline or high pH (7 to 12) *Vibrio cholerae.*

Bacteria are divided on the basis of osmotic pressure and salt concentration into:

1. Halophilic bacteria: requiring high salt concentration.

2. Osmophilic bacteria: requiring high osmotic pressure.

Culture Media

Culture

It is a method of growing microbes in nutritional media under controlled laboratory conditions. It is used to identify microbes and can also be used for antimicrobial susceptibility.

Culture media are used for recognition and identification (diagnosis) of microorganisms. The media are contained in plates (Petri dishes), in test tubes, flasks or screw capped bottles.

Types of culture media

1. Liquid (fluid) media e.g., Nutrient broth, Peptone water.

2. Semisolid media: e.g., motility media.

3. Solid media, e.g., Nutrient agar, MacConkey agar.

Solid media is used for the isolation of bacteria as pure culture. 'Agar' is most commonly used to prepare solid media. Agar is polysaccharide extract obtained from seaweed.

Solid media as agar in special purpose as:

1. Enriched media, simple media enriched with substances e.g., added blood 5-10%, added glucose 1-2%.

2. Selective media containing inhibitory substance as: e.g., bile salts, antibiotic, dyes,….etc., which favors the growth of the concerned microorganism and inhibit the growth of others, e.g., MacConkey agar, Bismuth Sulphate agar or SS agar.

3. Differential media, certain species produce characteristic growth that can easily recognized or can produce certain effects in the media, e.g., Triple sugar Iron agar (TSI), hemolytic and non-hemolytic species on blood agar.

4. Transport Medium.

Bacterial Growth Curve

During typical bacterial growth (growth cycle), bacteria cell divide by binary fission and their mass and number increase in an exponential manner. Bacterial growth in culture can be separated into at least four distinct phases:

1. Lag phase:

This is period of intense physiologic adjustment involving the induction of new enzymes and the synthesis and assembly of ribosome. There would be increase in

the size without any multiplication for same time. In lag phase and during this phase there occur:

1. increase in size of cells

2. increase in metabolic rate

3. Adaptation to new environment and necessary enzymes.

The length of lag phase depends upon

a. Type of bacteria.

b- Nutrients

c. Size or volume of inoculums.

d. Environmental factors like temperature and ph.

2. Logarithmic (Exponential) phase:

This phase is characterized by maximal rate of cell diction and followed by multiplication and increase in number of bacteria. The generation time (i.e., the time required for doubling the number of bacteria cell). During this phase is constant for given bacterial species grown under the same of condition but varies among species for example: the G.T. of Pseudomonas may be as brief as 14 minutes. The G.T. of M. tuberculosis as long 24 hours. In logarithmic phase the bacterial cell starts dividing and their number increase by geometric progression with time and during this period.

a. Bacteria have high rate of metabolism.

b. Bacteria are more sensitive to antibiotics.

c. Consumption of the medium depends on the concentration of material in the media.

3. Stationary phase:

After some time, growth rate becomes stationary and during this period there is a balance between cell growth and division and cell death. In stationary phase after some time a stage comes when rate of multiplication and death becomes almost equal it may be due to:

a. Depletion of nutrient.

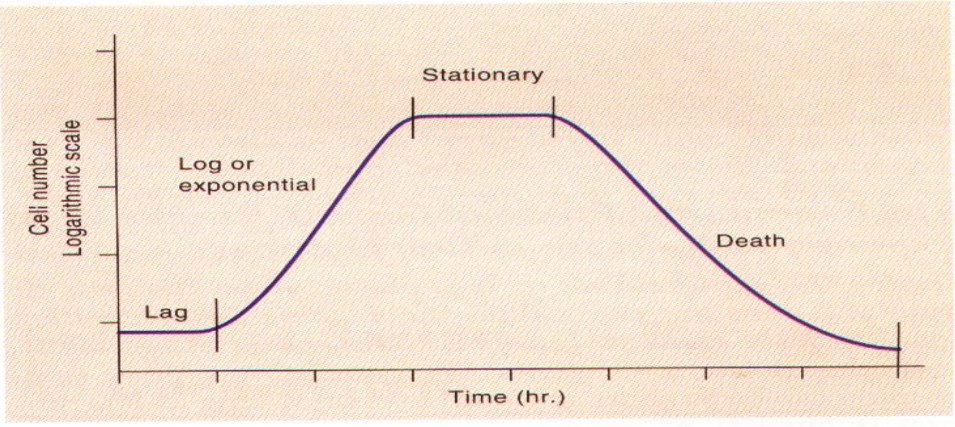
b. Accumulation of toxic products and sporulation may occur during this stage. Bacillus and Clostridium are capable of undergoing sporulation.

4. Decline or death phase:

During this phase bacterial cells undergo lyses which reduce the number of viable cells. In decline (death) phase, population decreases due to death of cells the factors responsible for that are:

a. Nutritional exhaustion , b. Toxic accumulation , c. Autolysis enzymes.

Fig. 2.2: Bacterial growth curve.



Control of Microorganisms by Physical and Chemical Agents

(STERILIZATION AND DISINFECTION)

INTRODUCTION

Sterilization and disinfection are essential for ensuring that medical and surgical instruments do not transmit infectious pathogens to patients .

Definitions of frequently used terms

Sterilization : Is the process by which all living cells, viable spores, and viruses are either destroyed or removed from an object or habitat , and is carried out in health-care facilities by physical or chemical methods.

Disinfection: Disinfection describes a process that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate objects.

Disinfectants: Disinfectants are agents, usually chemical, used to carry out disinfection and are normally used only on inanimate objects.

A disinfectant does not necessarily sterilize an object because viable spores and a few microorganisms may remain.

Antiseptics : Antiseptics are chemical agents applied to tissue to prevent infection by killing or inhibiting pathogen growth; they also reduce the total microbial population. Because they must not destroy too much host tissue, antiseptics are generally not as toxic as disinfectants.

Classification of Materials to be Sterilized / Disinfected

Critical Items

Critical items confer a high risk for infection if they are contaminated with any

microorganism. Thus, objects that enter sterile tissue or the vascular system must be sterile because any microbial contamination could transmit disease. This category includes surgical instruments, cardiac and urinary catheters, and implants etc.

Semi-critical Items

Semi-critical items contact mucous membranes or non-intact skin. This category includes respiratory therapy and anesthesia equipment, some endoscopes, laryngoscope blades, cystoscopes, and diaphragm fitting rings etc.

METHODS OF STERILIZATION

The various methods of sterilization are:

1. Physical Method

(a) Thermal (Heat) methods

(b) Radiation method

(c) Filtration method

2. Chemical Method

Physical Methods in Control

1. **Heat Sterilization:**

Fire and boiling water have been used for sterilization and disinfection since the time of the Greeks, and heating is still one of the most popular ways to destroy microorganisms. Either dry or moist heat may be applied.

a. Dry Heat Sterilization :

Many objects are best sterilized in the absence of water by dry heat sterilization. The items to be sterilized are placed in an oven at 160 to 170°C for 2 to 3 hours. Microbial death apparently results from the oxidation of cell constituents and denaturation of proteins.

Examples of dry heat sterilization are:

1.Incineration

2.Red heat

3.Flaming

4.Hot air oven

Dry Heat Sterilization advantages :

Dry heat does not corrode glassware and metal instruments as moist heat does, and it can be used to sterilize powders, oils, and similar items. Dry heat destroys bacterial endotoxins which are difficult to eliminate by other means and this property makes it applicable for sterilizing glass bottles which are to be filled aseptically.

Dry Heat Sterilization disadvantages :

dry heat sterilization is slow and not suitable for heat-sensitive materials like many plastic and rubber items.

b. Moist Heat Sterilization:

Moist heat readily kills viruses, bacteria, and fungi. Exposure to boiling water for 10 minutes is sufficient to destroy vegetative cells. Unfortunately the temperature of boiling water (100°C) is not high enough to destroy bacterial endospores that

may survive hours of boiling. Therefore boiling can be used for disinfection of drinking water and objects not harmed by water, but boiling does not sterilize.

Moist heat may be used in three forms to achieve microbial inactivation :-

1. Dry saturated steam – Autoclaving

2. Boiling water

3. Hot water below boiling point

Moist heat sterilization must be carried out at temperatures above 100°C in order to destroy bacterial endospores, and this requires the use of saturated steam under pressure. Steam sterilization is carried out with an **autoclave** , a device somewhat like a fancy pressure cooker, usually 121°C and pressure. At this temperature saturated steam destroys all vegetative cells and endospores in a small volume of liquid within 10 to 12 minutes. Treatment is continued for about 15 minutes to provide a margin of safety. Moist heat is thought to kill so effectively by degrading nucleic acids and by denaturing enzymes and other essential proteins. It also may disrupt cell membranes.

(b) Radiation method

Many types of radiation are used for sterilization like electromagnetic radiation (e.g. gamma rays and UV light) .The major target for these radiation is microbial DNA. Gamma rays cause ionization and free radical production while UV light causes excitation.

1. Ultraviolet (UV) radiation: Ultraviolet (UV) radiation kills all kinds of microorganisms due to its short wavelength (approximately from 10 to 400 nm) and high energy. The most lethal UV radiation has a wavelength of 260 nm, the wavelength most effectively absorbed by DNA. The primary mechanism of UV damage is the formation of thymine dimers in DNA.

Ultraviolet (UV) radiation around 260 nm is quite lethal but does not penetrate glass. Because of this disadvantage , UV radiation is used as a sterilizing agent only in a few specific situations. UV lamps are sometimes placed on the ceilings of rooms or in biological safety cabinets to sterilize the air and any exposed surfaces.

Because UV radiation burns the skin and damages eyes, people working in such areas must be certain the UV lamps are off when the areas are in use.

2. Ionizing radiation: Ionizing radiation is an excellent sterilizing agent and penetrates deep into objects. It will destroy bacterial endospores and vegetative cells, however, ionizing radiation is not always as effective against viruses. Gamma radiation is used in the cold sterilization of antibiotics, hormones, sutures, and plastic disposable such as syringes.

(c) Filtration method

Filtration process does not destroy but removes the microorganisms. It is used for both the clarification and sterilization of liquids and gases as it is capable of preventing the passage of both viable and non-viable particles. The major mechanisms of filtration are sieving, adsorption and trapping within the matrix of the filter material. Sterilizing grade filters are used in the treatment of heat sensitive injections and ophthalmic solutions, biological products and air and other gases for supply to aseptic areas. Membrane filters are used for sterility testing.

Chemical Methods in Control

Disinfectants are those chemicals that destroy pathogenic bacteria from inanimate surfaces. Some chemicals when used at appropriate concentration for appropriate duration can be used for sterilization and are called sterilant liquids.

Classification of disinfectants:

1. Based on consistency:

(a) Liquid (e.g., Alcohols, Phenols)

(b) Gaseous (Formaldehyde vapor)

2. Based on spectrum of activity:

(a) High level

(b) Intermediate level

(c) Low level

3. Based on mechanism of action:

(a) Action on membrane (e.g., Alcohol, detergent)

(b) Denaturation of cellular proteins (e.g., Alcohol, Phenol)

(c) Oxidation of essential sulfhydryl groups of enzymes (e.g., H2O2, Halogens)

(d) Alkylation of amino-, carboxyl- and hydroxyl group (e.g., Formaldehyde)

(e) Damage to nucleic acids (Formaldehyde)

Many different chemicals are available for use as disinfectants, and each has its own advantages and disadvantages:

Phenol

Mode of action: Act by disruption of membranes, precipitation of proteins and inactivation of enzymes.

Examples: 5% phenol, 1-5% Cresol, 5% Lysol , hexachlorophene, chlorhexidine, chloroxylenol (Dettol)

Applications: Phenol was the first widely used antiseptic and disinfectant. In 1867 Joseph Lister employed it to reduce the risk of infection during operations. They act as disinfectants at high concentration and as antiseptics at low concentrations.

They are bactericidal, fungicidal, mycobactericidal but are inactive against spores and most viruses.

Disadvantages: It is toxic, corrosive and skin irritant.

Alcohols

Mode of action: Alcohols dehydrate cells, disrupt membranes and cause coagulation of protein.

Examples: Ethyl alcohol, isopropyl alcohol and methyl alcohol.

Application: A 70% aqueous solution is more effective at killing microbes than absolute alcohols. some lipid-containing viruses are also destroyed. 70% ethyl alcohol (spirit) is used as antiseptic on skin. Isopropyl alcohol is preferred to ethanol. It can also be used to disinfect surfaces. It is used to disinfect clinical thermometers. Methyl alcohol kills fungal spores, hence is useful in disinfecting inoculation hoods.

Disadvantages: Skin irritant, volatile (evaporates rapidly), inflammable.

Aldehydes

Mode of action: Acts through alkylation of amino-, carboxyl- or hydroxyl group, and probably damages nucleic acids. It kills all microorganisms, including spores.

Examples: Formaldehyde, Gluteraldehyde.

Application: 40% Formaldehyde (formalin) is used for surface disinfection and fumigation of rooms, chambers, operation theatres, biological safety cabinets, wards, sick rooms etc.

Disadvantages: Vapors are irritating (must be neutralized by ammonia), has poor penetration, leaves non-volatile residue, activity is reduced in the presence of protein.

Halogens

Mode of action: They are oxidizing agents. Chlorine reacts with water to form hypochlorous acid, which is microbicidal. The result is oxidation of cellular materials and destruction of vegetative bacteria and fungi, although not spores.

Examples: fluorine, chlorine, bromine, iodine, and astatine.

Applications: Tincture of iodine (2% iodine in 70% alcohol) is an antiseptic. 10% Povidone Iodine is used undiluted in pre and postoperative skin disinfection. In higher concentrations chlorine is used to disinfect swimming pools. 0.5% sodium hypochlorite is used in serology and virology. Used at a dilution of 1:10 in decontamination of spillage of infectious material.

Disadvantages: They are rapidly inactivated in the presence of organic matter. Iodine is corrosive and staining.

Heavy Metals

Mode of action: Act by precipitation of proteins and oxidation of sulfydryl groups. They are bacteriostatic.

Examples : Ions of heavy metals such as mercury, silver, arsenic, zinc, and copper.

Applications : 1% silver nitrate solution can be applied on eyes as treatment for ophthalmia neonatorum . Silver sulphadiazine is used topically to help to prevent colonization and infection of burn tissues. Mercurials are active against viruses at dilution of 1:500 to 1:1000. Copper salts are used as a fungicide.

Disadvantages: Mercuric chloride is highly toxic, are readily inactivated by organic matter.

Surface Active Agents

Mode of actions:

They disrupt microbial membranes and may also denature proteins .These compounds have long chain hydrocarbons that are fat soluble and charged ions that are water-soluble.

Examples: Soaps or detergents. Detergents can be anionic or cationic. Detergents containing negatively charged long chain hydrocarbon are called anionic detergents. These include soaps and bile salts. If the fat-soluble part is made to have a positive charge by combining with a quaternary nitrogen atom, it is called cationic detergents. Cationic detergents are known as quaternary ammonium compounds .Cetrimide and benzalkonium chloride act as cationic detergents.

Application:

They are active against vegetative cells, Mycobacteria and enveloped viruses. They are widely used as disinfectants at dilution of 1-2% for domestic use and in hospitals.

Disadvantages: Their activity is reduced by hard water, and organic matter. Pseudomonas can metabolize cetrimide, using them as a carbon, nitrogen and energy source.

Hydrogen Peroxide

Mode of action: It acts on the microorganisms through its release of nascent oxygen. Hydrogen peroxide produces hydroxyl-free radical that damages proteins and DNA.

Application:

It is used at 6% concentration to decontaminate the instruments, equipment's such as ventilators. 3% Hydrogen Peroxide Solution is used for skin disinfection and deodorising wounds and ulcers. Strong solutions are sporicidal.

Disadvantages:

Decomposes in light, broken down by catalase, proteinaceous organic matter drastically reduces its activity.

Mechanisms of Action of Antimicrobial Agents

Modern medicine is dependent on chemotherapeutic agents, chemical agents that are used to treat disease.

Chemotherapeutic agents destroy pathogenic microorganisms or inhibit their growth at concentrations low enough to avoid undesirable damage to the host. Most of these agents are antibiotics, microbial products or their derivatives that can kill susceptible microorganisms or inhibit their growth.

Antimicrobial drugs can damage pathogens in several ways, as can be seen in **table (3)** which summarizes the mechanisms of the antibacterial drugs :

Table 3: Mechanisms of Antibacterial Drug Action.

	Target \ Drug	Mechanism of Action
1.	= Cell Wall Synthesis Inhibition Penicillin Ampicillin Carbenicillin Methicillin Cephalosporins	Inhibit the bacterial cell wall peptidoglycan. Activate cell wall lytic enzymes.
2.	= Protein Synthesis Inhibition Streptomycin Tetracyclines	Binds with the 30S subunit of the bacterial ribosome to inhibit protein synthesis.
	Erythromycin and clindamycin	Bind to the 50S ribosomal subunit and inhibit protein synthesis
3.	= Nucleic Acid Synthesis Inhibition Ciprofloxacin and other quinolones	Inhibit bacterial DNA gyrase and thus interfere with DNA replication, transcription, and other activities involving DNA.
	Rifampin	Blocks RNA synthesis by binding to and inhibiting the DNA-dependent RNA polymerase.
4.	= Cell Membrane Disruption Polymyxin B	Binds to the plasma membrane and disrupts its structure and permeability properties.

Gram Positive Cocci Bacteria
Genus : *Staphylococcus*

Staphylococcus : General Characteristics

Staphylococci are Gram-positive cocci, 1 micrometer in diameter, non-motile, non-spore- forming, non-capsulated and facultative anaerobic. The name *Staphylococcus* is derived from the Greek term „staphyle“, meaning „a bunch of grapes“. This name refers to the fact that the cells of these Gram-positive cocci grow in a pattern resembling a cluster of grapes as a result of division in many planes during replication. However, microorganisms in clinical material may also appear as single cell or pairs. On nutrient agar they form colonies, white, yellow-golden in color. Their hemolytic capacity is variable. Pathogenic strains produce coagulase, ferment sugar (glucose, lactose, mannitol) with acid production and catalase-producing .

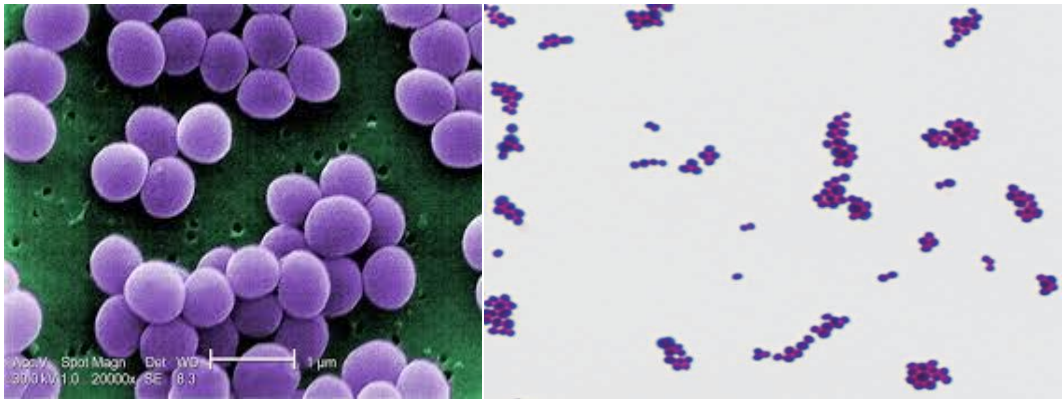


Figure 4-1: Staphylococcus aureus showing gram-positive cocci in pairs, tetrads, and clusters.

The genus *Staphylococcus* contains about 50 species and subspecies today. Only some of them are important as human pathogens:

– *Staphylococcus aureus,*

– *Staphylococcus epidermidis*

– *Staphylococcus hominis*

– *Staphylococcus haemolyticus*

– *Staphylococcus saprophyticus*

The genus *Staphylococcus* can be divided into two subgroups **(on the basis of its ability to clot blood plasma by enzyme coagulase):**

1- Coagulase-positive :-e g. -*Staphylococcus aureus*

2- Coagulase-negative:-e g. -*Staphylococcus epidermidis*

-*Staphylococcus saprophyticus*

Also the genus *Staphylococcus* can be divided into three groups (on the basis of pigment production):

1. *Staphylococcus aureus* : Most virulent species. It is responsible for a wide range of medical illnesses extending from mild localized skin infection to life threatening septicemia. Colonies golden-yellow in culture media.

2. *Staphylococcus epidermidis* : (*Staph. albus*) white colonies causes opportunistic infections in debilitated or immunocompromised patients.

3. *Staphylococcus saprophyticus* : (*Staph. citrus*). Lemon-yellow causes opportunistic infection, common cause of UTI in women.

The species *Staphylococcus aureus*

Morphology

– Gram-positive, spherical cells, mostly arranged in irregular grape like clusters.

– The peptidoglycan layer is the major structural component of the cell wall. It is important in the pathogenesis of staphylococcal infections. Other important component of cell wall is teichoic acid.

– Protein A is the major protein component of the cell wall. It is located on the cell surface but is also released into the culture medium during the cell growth. A unique property of protein A is its ability to bind to the Fc part of all IgG molecules to block phagocytosis .

Staphylococcus aureus culture characteristics

1-Colonies on solid media are round, regular, smooth, slightly convex and 2 to 3 mm in diameter after 24h incubation.

2- Most strains show a ß-hemolysis surrounding the colonies on blood agar.

3- *S. aureus* cells produce cream, yellow or orange pigment.

4- Salt tolerant (8-10% NaCl): allows them to tolerate the salt present on human skin.

5- Like most of medical important non-spore-forming bacteria, *S. aureus* is rapidly killed by temperature above 60 °C.

6- *S. aureus* is susceptible to disinfectants and antiseptics commonly used.

7- *S. aureus* can survive and remain virulent long periods of drying especially in an environment with pus.

8- Produce Beta-lactamase : confers resistance (antibiotic resistance).

Virulence Factors of *Staphylococcus aureus* (Staphylococcal toxins and enzymes)

(A). Staphylococcal toxins

Staphylococcus aureus produces many virulence factors, including the following cytolytic or membrane-damaging toxins:

(1) – alpha toxin

(2) – beta toxin } RBCs hemolysin

(3) – gamma toxin : The γ hemolysin is a leukocidin that lyses white blood cells

(4) – Exfoliative toxins: Staphylococcal scalded skin syndrome (SSSS), a spectrum of diseases characterized by exfoliative dermatitis.

(5) – Eight enterotoxins (A-E , G- I) : Staphylococcal food poisoning due to production of enterotoxins . The enterotoxins are stable to heating at 100 °C for 30 minutes and are resistant to hydrolysis by gastric and jejunal enzymes. Enterotoxin A is most commonly associated with disease. Enterotoxins C and D

are found in contamined milk products, and enterotoxin B causes staphylococcal pseudomembranous enterocolitis.

(6) – Toxic Shock Syndrome Toxin 1 (TSST-1) : TSST-1, formerly called pyrogenic exotoxin C and enterotoxin F, is a heat and proteolysis resistant , chromosomally mediated exotoxin. High fever, , diarrhea , shock and erythematous skin rash which desquamate . Death in patients with TSS is due to hypovolemic shock leading to multi organ failure.

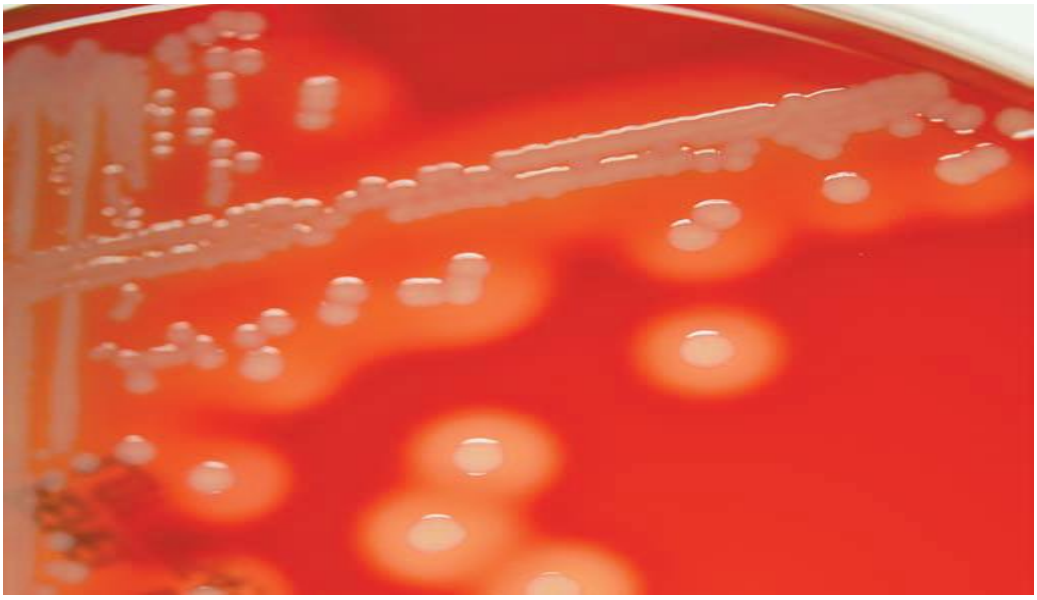


Figure 4-2 Colonies of *Staphylococcus aureus* on a blood agar plate after 24 hours incubation. The colonies are surrounded by clear zones of hemolysis about 1 cm in diameter.

(B). Staphylococcal enzymes

Staphylococcus aureus strains produce several other extracellular, biologically active substances, including :

(1) Coagulase :

S. aureus strains possess two forms of coagulase:

A – bound : Coagulase bound to the staphylococcal cell wall can directly convert fibrinogen to insoluble fibrin and cause the staphylococci to clump.

B – free: The cell-free coagulase accomplishes the same result by reacting with a globulin plasma factor.

Coagulase may cause the formation of fibrin layer around a staphylocccal abscess, thus localizing the infection and protecting the organisms from phagocytosis.

(2) Catalase :

All staphylococci produce catalase, which catalyzes the conversion of toxic hydrogen peroxide to water and oxygen. Hydrogen peroxide can accumulate during bacterial metabolism or after phagocytosis.

(3) Hyaluronidase: Hyaluronidase hydrolyzes hyaluronic acid, the acidic mucopolysaccharides present in the a cellular matrix of connective tissue. This enzyme facilitates the spread of *S. aureus* in tissues. More than 90% of *S. aureus* strains produce this enzyme.

(4) Fibrinolysin : Fibrinolysin, also called staphylokinase, is produced by virtually all *S. aureus* strains and can dissolve fibrin clots.

(5) Beta-lactamase : confers resistance (antibiotic resistance)

(6) DNase: degrades DNA

Staphylococcus aureus pathogenicity

S. aureus is pathogenic for human as well as for all domestic and free-living warm-blooded animals. *S. aureus* causes disease through the production of toxin or through direct invasion and destruction of tissue.

The clinical manifestations of some staphylococcal diseases are almost exclusively the result of toxin activity (e.g. staphylococcal food poisoning and TSS), whereas other diseases result from the proliferation of the staphylococci, leading to abscess formation and tissue destruction (e.g. cutaneous infection, endocarditis, pneumonia, osteomyelitis, septic arthritis). Bacteremia, septicemia, and deep organ infection (e.g., brain, kidney, lung).

Diagnostic Laboratory Tests

A. Specimens

Surface swab pus or aspirate from an abscess, blood, tracheal aspirate, or spinal fluid for culture, depending on the localization of the process, are all appropriate specimens for testing. The anterior nares are frequently swabbed to determine nasal colonization, either by culture or nucleic acid amplification tests, for epidemiological purposes.

B. Smears

Typical staphylococci appear as gram-positive cocci in clusters in Gram-stained smears of pus or sputum. It is not possible to distinguish saprophytic *(S. epidermidis)* from pathogenic *(S. aureus)* organisms on smears.

C. Culture

Specimens planted on blood agar plates give rise to typical colonies in 18 hours at 37°C, but hemolysis and pigment production may not occur until several days later and are optimal at room temperature. *S. aureus* but not other staphylococci ferment mannitol. Specimens contaminated with a mixed flora can be cultured on media containing 7.5% NaCl; the salt inhibits most other normal microbiota but not *S. aureus*. Mannitol salt agar or commercially available chromogenic media are used to screen for nasal carriers of *S. aureus* and patients with cystic fibrosis.

D. Catalase Test

E. Coagulase Test

Treatment

90 % of of *S. aureus* are resistant to penicillin G. Most of these strains produce beta - lactamase . Such organisms can be treated with beta lactamase - resistant penicillins (e. g. nafcillin or cloxacillin) , some cephalosporins, or vancomycin. Treatment with a combination of beta - lactamase- sensitive penicillin (e. g. amoxicillin) and a beta -lactamase inhibitor (clavulanic acid) is also useful .

Gram Positive Cocci Bacteria
Genus : *Streptococcus*

Introduction

The streptococci are gram-positive spherical bacteria that characteristically form pairs or chains during growth, Catalase negative. They are widely distributed in nature. Some are members of the normal human flora others are associated with important human diseases. Streptococci elaborate a variety of extracellular substances and enzymes. The streptococci are a large and heterogeneous group of bacteria and understanding the classification of it is a key to understanding their medical importance.

The most pathogenic species for human:

1- *Streptococcus pyogenes*. (it can cause : Pharyngitis, rheumatic fever, glomerulonephritis).

2 - *Streptococcus pneumoniae*. (it can cause: Pneumonia, meningitis, endocarditis).

3 - Viridans Streptococci include : (*S. mitis* it can cause endocarditis.,
S. mutans is the cause of dental caries).

4 - *Streptococcus bovis.* (it can cause endocarditis).

5 - *Streptococcus agalactiae.* (it can cause Neonatal sepsis and meningitis).

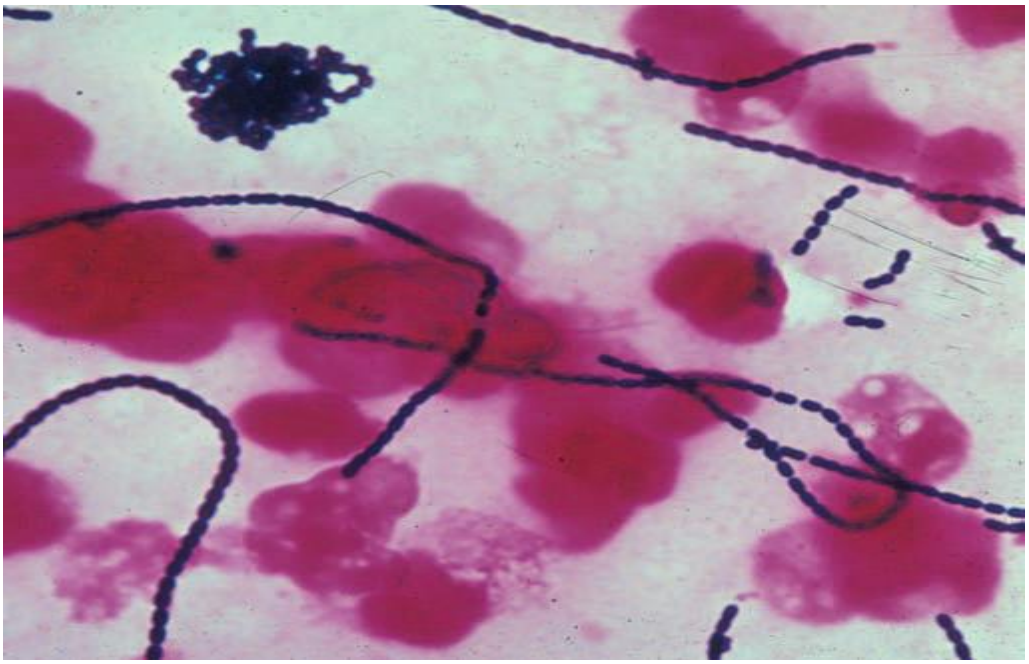


Figure 14-1 Streptococci grown in blood culture showing gram-positive cocci in chains.

Classification

1-Beta-hemolytic group: causes complete lysis of RBC

2-Alpha-hemolytic group: causes partially lysis of RBC

3-Gamma-hemolytic group: does not cause RBC lysis

Streptococcus can also be classified on the basis of antigen C (carbohydrate) present on the cell wall called Lancefield antigen. There are more than 50 types, but five are important and cause diseases in humans. They are grouped A, B, C, D, F, and G.

1-Beta-hemolytic group: causes complete lysis of RBC

It includes Lancefield group A, B, C, F, and G.

(A = *S. pyogenes*, B = *S. agalactiae*, C -=*S. equi*, F, G = *S. dysgalactiae*).

- Group A / *Streptococcus pyogenes (pus forming)*

Streptococcus pyogenes is a prototypical human pathogen. *S. pyogenes* is the main human pathogen associated with local or systemic invasion and poststreptococcal immunologic disorders. *S. pyogenes* typically produces large (1 cm in diameter) zones of beta hemolysis around colonies. They are Bacitracin sensitive and catalase negative.

Figure 5-1: Group A β-hemolytic streptococci (*Streptococcus pyogenes*) after growth overnight on a 10-cm plate with 5% sheep blood agar. The small (0.5–1 mm diameter) white colonies are surrounded by diffuse zones of β-hemolysis 7–10 mm in diameter.

Virulence factors of *Streptococcus pyogenes*

More than 20 extracellular products that are antigenic are elaborated by *S. pyogenes*, including the following:

1- Streptokinase (Fibrinolysin)

Streptokinase is produced by many strains of group A beta -hemolytic streptococci. It transforms the plasminogen of human plasma into plasmin, an active proteolytic enzyme that digests fibrin and other proteins.

2- Streptodornase

Streptodornase (streptococcal deoxyribonuclease) depolymerizes DNA. The enzymatic activity can be measured by the decrease in viscosity of known DNA solutions.

3- Hyaluronidase

Hyaluronidase splits hyaluronic acid, an important component of the ground substance of connective tissue. Thus, hyaluronidase aids in spreading infecting microorganisms (spreading factor). Hyaluronidases are antigenic and specific for each bacterial or tissue source.

4- Pyrogenic Exotoxins (Erythrogenic Toxin)

Pyrogenic exotoxins are elaborated by *S. pyogenes*. There are three antigenically distinct streptococcal pyrogenic exotoxins: A, B, and C. Exotoxin A has been most widely studied. The streptococcal pyrogenic exotoxins have been associated with streptococcal toxic shock syndrome and scarlet fever. The pyrogenic exotoxins act as superantigens, which stimulate T cells .

5- Hemolysins

The beta-hemolytic group A *S. pyogenes* elaborates two hemolysins (streptolysins). Streptolysin O and Streptolysin S , is the agent responsible for the hemolytic zones around streptococcal colonies growing on the surface of blood agar plates.

Clinical significance

- Strep throat
- Dental infection
- Skin infections, impetigo, erysipelas, cellulitis, and necrotizing fasciitis
- Streptococcal toxic shock syndrome
- Scarlet fever / starts with fever, sore throat, strawberry tongue, and rashes on trunk, which spread all over the body and feel like rough sandpaper; symptoms last for 2-5 days .

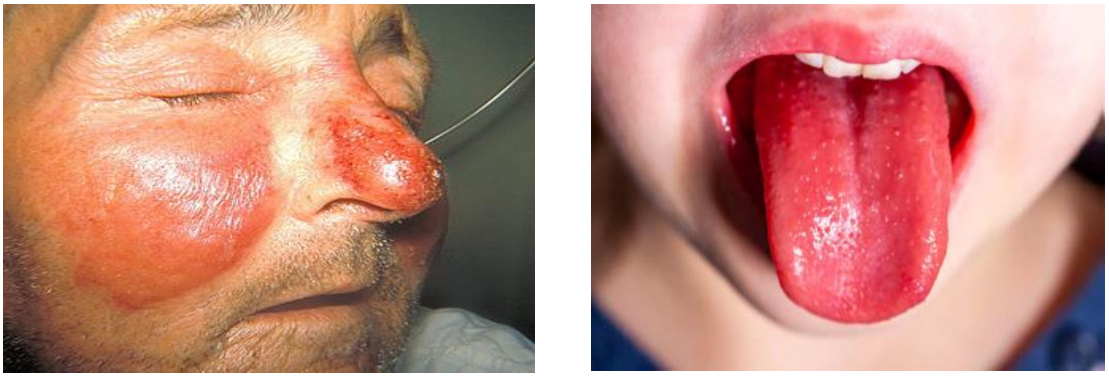


FIGURE 5.2. 1-Erysipelas of the face due to invasive Streptococcus, 2- Strawberry tongue (scarlet fever).

Postinfectious sequelae

- **Rheumatic fever (RF)**
- RF is a postinfectious sequela after strep throat but not after skin infection; there are about 3% chances in the untreated population that they may develop acute rheumatic fever
- RF is an autoimmune, antibody-mediated (against M antigen) disorder that cross reacts with hosts' own tissue
- **Poststreptococcal glomerulonephritis (PSGN)**
- May occur one or 2 weeks after the initial infection of either strep throat or skin infection.

Treatment

All *S. pyogenes* are susceptible to penicillin G, and most are susceptible to erythromycin. Some are resistant to tetracyclines. Antimicrobial drugs have no effect on established glomerulonephritis and rheumatic fever.

2-Alpha-hemolytic group: causes partially lysis of RBC

- Partially hemolyzes the blood agar
- Forms green color colony
- No Lancefield groups

Includes:

a) Viridans streptococcus group (*S. mitis* it can cause endocarditis.,

S. mutans is the cause of dental caries).

b) *Streptococcus pneumonia*

Gram positive, alpha-hemolytic, diplococci, lancet-shaped bacteria in chains or pairs.

- Lysis by bile and optochin sensitive
- There are more than 90 serotypes, but few cause serious infections.

Virulence factors:

- Capsule ,Adherence protein
- Biofilm formation , Pneumolysin toxin

Clinical significance:

- Pneumonia (lobular)
- Otitis media in children
- Meningitis
- Septic arthritis
- Pneumococcal endocarditis

Choice of antibiotics:

- First choice: penicillin G, ceftriaxone, levofloxacin, vancomycin
- Second choice: clindamycin, cefepime, doxycycline

3- Gamma-hemolytic group

Does not cause any hemolysis on blood agar; comprises:

Enterococcus (Includes: *Enterococcus faecalis* and *Enterococcus faecium*

Hospital-acquired urinary tract infection- Wound infection).

b) Nonenterococcus (Includes *S. bovis* and *S. equinus* ; still a part of streptococcus classification group D causes endocarditis and biliary tract infections).

Gram Negative Cocci Bacteria
Genus : Neisseria and Moraxella

Genus : Neisseria

General characteristic

Neisseria are Gram-negative cocci that usually occur in pairs, Kidney bean shape. *Neisseria gonorrhoeae* (gonococci) and *Neisseria meningitidis* (meningococci) are pathogenic for human , some *Neisseria* are normal inhabitants of the human respiratory tract . Meningococci and Gonococci need 5-10% CO2 and require chocolate agar to grow on. Meningococci have polysaccharide capsules, whereas gonococci do not . Meningococci typically are found in the upper respiratory tract and cause meningitis, while gonococci cause genital infections.

Neisseria gonorrhoeae (gonococcus):

Important properties:

• Gram negative cocci in pairs (diplococci) and kidney shape.
• It is non-capsulated.
• It is non-motile bacteria.
• Aerobic or facultative anaerobes.

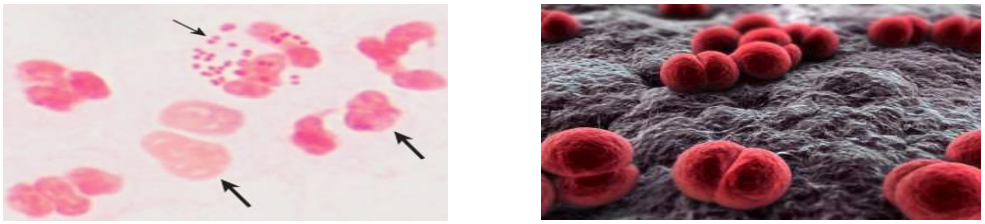


FIGURE6.1: ***Neisseria*** diplococci and kidney shape

Antigenic structure and Virulence factors:

- Endotoxin is lipooligosaccharide(LOS). The toxicity of gonococcal infections is largely due to endotoxic LOS effect.
- IgA protease: it mediates attachment to cells by hydrolysis of IgA at mucous membranes.
- Type IV pili: Play an important role in attaching to human mucosal epithelial cells, fallopian tube, and vaginal epithelial cells. Pili also help the bacteria for motility.

Source and transmission :

• Natural habitat is genital tract of human only(obligate human pathogen).

• The organism is usually transmitted from person to person by sexual contact.

• Also the organism can be transmitted from infected mother to her newborn during birth.

Diseases and clinical findings:

1. Venereal diseases (VD):-

a-Genital VD (gonorrhea): *Neisseria gonorrhoeae* causes gonorrhea

• In males are characterized by urethritis with yellow and creamy pus (purulent discharge) , and painful urination with accompanied by dysuria. The process may extend to prostate and epididymis. Untreated infection, fibrosis occurs, sometime leading to urethral stricture and infertility.

• In females , the primary infection is in endocervix and extends to urethra (urethritis) and vagina (vaginitis) , causing a purulent vaginal discharge and

cervicitis. It may then progress to uterine tube , causing salpingitis and fibrosis , which can result in sterility or ectopic pregnancy.

b-Extra-genital VD:

• Proctitis occurs in both sexes .

• Ophthalmia neonatorum in newborns is acquired from infected birth canal of mother, untreated infection result in blindness. To prevent the infection , instillation of tetracycline , erythromycin, or silver nitrate into conjunctival sac of newborn.

2. Non-venereal disease (as complications):

Disseminated gonococcal infection (DGI) is commonly after blood invasion for primary infection may lead to lesions like endocarditis, arthritis, meningitis and pelvic - inflammatory disease (PID).

Lab. Diagnosis:

• Microscopic examination of genital discharge: Gram-stained smear reveal many Gram negative intracellular diplococci.

• The specimens should be immediately cultured on Thayer-Martin medium and incubated under 5-10% CO2 condition at 35-37C.

• Serologic test such as : ELISA .

• Nucleic acid technique by (PCR).

Choice of antibiotic

- First choice: Ceftriaxone + azithromycin or doxycycline
- Second choice: Spectinomycin + azithromycin, gentamicin + azithromycin
- No vaccine is available

Prevention:

a. Safe sex practice and use of condom (sex hygiene).

b. No effective vaccine is available.

Neisseria meningitidis (meningococcus):

Important properties:

- Gram-negative diplococci, obligate aerobic bacteria, nonspore forming, and nonmotile
- Catalase and oxidase positive, and grows on Thayer-Martin agar
- It ferments maltose which differentiates it from *Neisseria gonorrhoeae* Causes meningitis, sepsis, and septic shock in infants, children, and young adults
- About 5% of people are asymptomatic carriers, harvesting the bacteria in the nasopharynx area
- It is capsulated.

Virulence factors:

- Capsule: meningococcus has a polysaccharide capsular that enable the organism to resist phagocytosis.
- Endotoxin (LPS): is responsible for many of toxic effects found in meningococcal disease.
- Toxins: IgA protease that destroys immunoglobulin A. IgA plays an important role in protecting the host from invading bacteria Pili and Outer Membrane Proteins (OMP).

- Produce catalase that breaks hydrogen peroxide molecule inside the neutrophils.

Source and transmission:

• The natural reservoir of meningococcus is only human nasopharynx.

• The organisms are transmitted by airborne droplets to other persons.

Diseases and Clinical features:

The meningococcus causes meningitis only in human. Meningococcus is a common cause of meningitis in children (3 months to 1 year old). and among young adult .

Treatment:

- First choice: ceftriaxone + dexamethasone

- Penicillin G IV and ampicillin IV

- Second choice : ciprofloxacin or levofloxacin, chloramphenicol, meropenem, aztreonam.

Genus : Moraxella

Moraxella catarrhalis:

- Gram-negative diplococci morphologically resemble Neisseria

- Does not grow in Thayere-Martin agar

- Colonizes upper respiratory tract in 40%-50% of normal school children

- Causes otitis media in young children (third most common cause) and second most common cause after *Haemophilus influenza*. It is also responsible for pneumonia in elderly patients.

- Many strains produce B-lactamase

Reservoir: Upper Respiratory Tract

Transmission: Respiratory droplets

Pathogenesis:

Moraxella catarrhalis causes lower respiratory infection in adults with chronic lung disease and is a common cause of otitis media, sinusitis, and conjunctivitis in children. Causes pneumonia, bronchitis, tonsillitis, bacteremia and septicemia.

Treatment

Moraxella catarrhalis can be treated with antibiotics (Amoxicillin-clavulanic acid) . The great majority of clinical isolates of this organism produce beta-lactamases, so are resistant to penicillin , ampicillin, and amoxicillin.

Spore-Forming Gram-Positive Bacilli

A- Strict aerobic – Genus *Bacillus.*

B- Strict anaerobic – Genus *Clostridium.*

Anaerobic bacteria / *Clostridium*

Clostridium and *bacillus* are both spore-forming. *Clostridium* is anaerobic and *bacillus* is aerobic. *Clostridium* are large rods, G +ve, anaerobic produce toxins & enzymes, spore forming & some species are motile. *Clostridium* are found in soil especially in soil fertilized with animal excreta . The genus *clostridium* includes over 50 species , the most common pathogens are :

***1. Clostridium perfringens* :** cause gas gangrene, food poisoning.

2. *Clostridium tetani* : cause tetanus.

3. *Clostridium botulinum* : cause botulism.

4. *Clostridium difficile* : cause toxic enterocolitis. (Antibiotic – Associated Diarrhea).

Clostridium perfringens

C. perfringens causes two distinct diseases, gas gangrene and food poisoning, depending on the route of entry into the body.

Disease: Gas Gangrene

Gas gangrene (myonecrosis, necrotizing fasciitis) is one of the two diseases caused by *C. perfringens.*

Transmission

Spores are located in the soil; vegetative cells are members of the normal flora of the colon and vagina. Gas gangrene is associated with war wounds, automobile and motorcycle accidents, and septic abortions (endometritis).

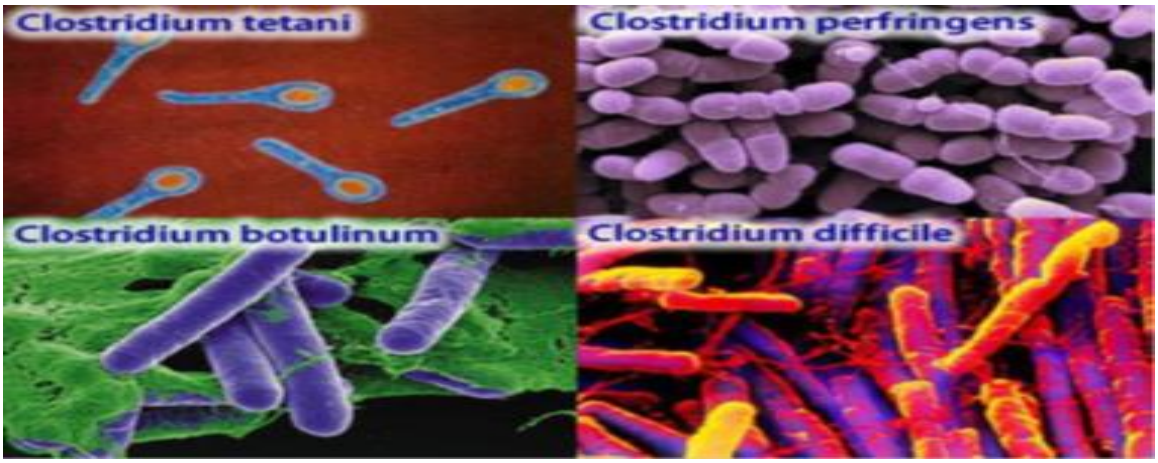


FIGURE 7.1: *clostridium* species.

Pathogenesis

Organisms grow in traumatized tissue (especially muscle) and produce a variety of toxins. The most important is alpha toxin (lecithinase), which damages cell membranes, including those of erythrocytes, resulting in hemolysis. Degradative enzymes produce gas in tissues.

Clinical Findings

Pain, edema, and cellulitis occur in the wound area. Crepitation indicates the presence of gas in tissues. Shock and death can ensue. Mortality rates are high.

Treatment and Prevention

Penicillin G is the antibiotic of choice. Wounds should be cleansed and debrided. There is no vaccine.

Disease: Food Poisoning

Food poisoning is the second disease caused by *C. perfringens.*

Transmission

Spores are located in soil and can contaminate food. The heat-resistant spores survive cooking and germinate. The organisms grow to large numbers in reheated foods, especially meat dishes.

Pathogenesis

C. perfringens is a member of the normal flora in the colon but not in the small bowel, where the enterotoxin acts to cause diarrhea.

Clinical Findings

The disease has an 8- to 16-hour incubation period and is characterized by watery diarrhea with cramps and little vomiting. It resolves in 24 hours.

Clostridium tetani:

Clostridium tetani which causes tetanus is worldwide in distribution in the soil and in the feces of animals and human. Produce neurotoxin, tetanospasmin. Small amounts of toxin can be lethal for humans. The vegetative cells of *C. tetani*

produce the toxin tetanospasmin which binds to motor neurons that effect to the spinal cord and brain.

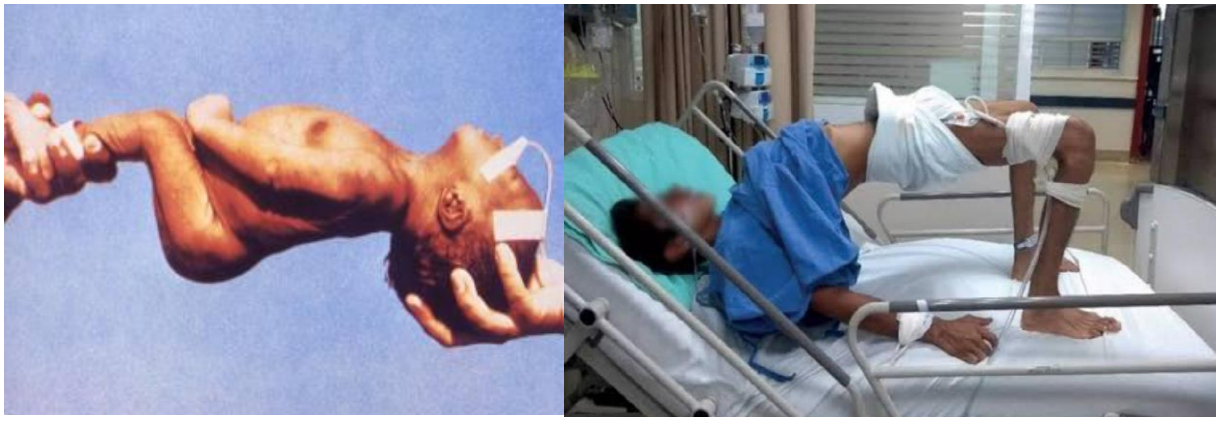


FIGURE 7.2: *Tetanus patient.*

Symptoms:

Tetanus symptoms usually emerge about 7 to 10 days after initial infection. However, this can vary from 4 days to about 3 weeks. Stiffness usually starts with the chewing muscles, hence the name lockjaw, then spread to the neck and throat, causing difficulties with swallowing and breathing difficulties. In severe cases, the spine will arch backward as the back muscles become affected. This is more common when children experience a tetanus infection.

Pathogenesis:

C. tetani is not invasive organism. The infection remains localized in the area of tissue (wound, burn, injury) in which the spores have been introduced, and the disease is entirely a toxemia. Germination of the spore and development of vegetative that produce toxin are aided by necrotic tissue, and pyogenic infections,

and then the toxin released reaches the central nervous system (CNS) and rabidly become fixed to receptors in the spinal cord and brain.

Treatment:

Intramuscular administration antitoxin (tetanus immune globulin) to neutralizes the toxin that has not been fixed to nervous tissues. Patients who develop symptoms of tetanus should receive muscle relaxants, sedation, and assisted ventilation. Sometimes surgical to removes the necrotic tissue. Penicillin inhibits the growth of *C. tetani* and stops toxin production.

Prevention of tetanus depends on:

1. Active immunization with toxoids.
2. Care of wounds contaminated with soil, etc.
3. Prophylactic use of antitoxin
4. Administration of penicillin

Aerobic Spore-forming Gram-positive bacilli

(*Bacillus anthracis* , *B. cereus*)

- *Bacillus anthracis* :

- Rod shape bacteria causing anthrax, a zoonotic disease, and usually infects animals but occasionally can be transferred to humans
- Word anthrax is a Greek word meaning coal, which refers to a dark color of skin eschars it causes
- Spore-forming bacteria survive in a wide variety of environments and a wide range of temperatures.

- **Virulence factors:**

- Capsule: helps the bacteria escape from phagocytic cells.
- Toxins.

- **Clinical significance:**

- Responsible for anthrax.

Anthrax

Anthrax usually occurs in domestic animals such as cows, goats, and horses ; humans are infected when exposed to infected animals or their products; anthrax can be classified into the following types:

A-Cutaneous anthrax-Hide-porters disease : In dermal anthrax the pathogens enter through injuries in the skin. A local infection is similar to necrotic ulcer .

B. Pulmonary anthrax-Wool sorters disease : Wool sorter's disease results from inhalation of dust containing the pathogen.

C. Intestinal anthrax : Ingestion of contaminated foods can result in intestinal.

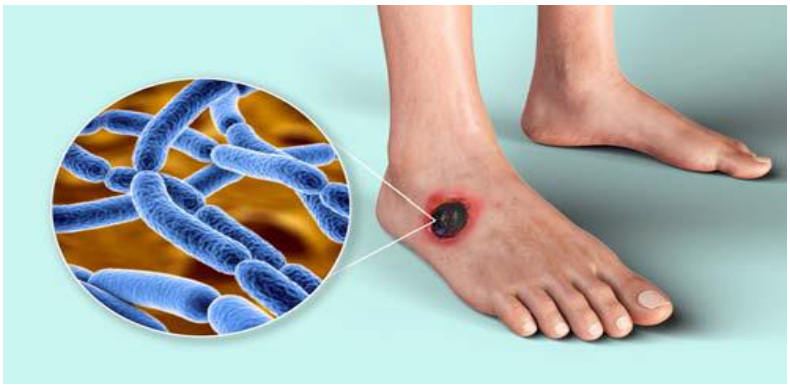


FIGURE 7.3: *Cutaneous anthrax.*

Treatment:

Antibiotics:

- First choice
    - Ciprofloxacin
    - Penicillin or ampicillin
- Second choice
    - Levofloxacin
    - Doxycycline
    - Clindamycin

Prevention of tetanus depends on:

- Proper hygiene and protection from infected animals
- Active immunization in high-risk patients
- BioThrax is the Food and Drug Administration (FDA)-approved toxin base vaccine.

- *Bacillus cereus*:

- Spore-forming, Gram-positive bacilli isolated from soil, vegetables, milk, fried rice, cooked poultry, meat, soup, fruits.
- It is also found in the gut flora of invertebrates
- Gastroenteritis, caused by the production of enterotoxins

- Virulence factors:

- There are two types of enterotoxin :

1- Heat-stable: manifested by nausea, vomiting, abdominal cramp, and rarely

diarrhea; it is self-limiting; usually, by consumption of contaminated rice where heat-stable toxin is not destroyed; incubation time is 1e6 hours

2- Heat-labile: usually causes profuse watery diarrhea and abdominal cramps it results from ingestion of contaminated meat and vegetables it has longer incubation time.

- Pathogenesis :

B. cereus is an important cause of eye infections, such as severe keratitis, endophthalmitis, and panophthalmitis. Typically, the organisms are introduced into the eye by foreign bodies associated with trauma. *B. cereus* has also been associated with localized infections and with systemic infections, including endocarditis, meningitis, osteomyelitis, and pneumonia; the presence of a medical device or intravenous drug use predisposes to these infections. *B. cereus* is resistant to a variety of antimicrobial agents, including penicillins and cephalosporins. Serious non–foodborne infections should be treated with vancomycin or clindamycin with or without an aminoglycoside.

Aerobic Non-Spore-Forming Gram-Positive Bacilli
(*Corynebacterium diphtheria* , *Listeria monocytogenes*)
and
ACID-FAST BACILLI
(Mycobacterium tuberculosis)

Corynebacterium diphtheria :-

Corynebacteria are club-shape, gram positive, non-spore forming bacteria, when cluster together seems to be arranged in characteristic pattern resemble like a Chinese letters. Aerobic and non-motile. *Corynebacterium diphtheria* is the most important member of the group, as it can produce powerful Exotoxins that cause diphtheria in humans. *C. diphtheria* are 0.5-1 micro millimeters in diameter, bacilli irregular swelling at one end that give them club-shaped (clubbed end), C diphtheriae can grow on the mucous membranes of the upper respiratory tract or in minor skin wounds. *C diphtheriae* and other corynebacteria grow aerobically on most ordinary laboratory media. On Loeffler serum medium, corynebacteria grow much more readily than other respiratory organisms, and the morphology of organisms is typical in smears made from these colonies.

Pathogenesis:

C. diphtheria is pathogenic bacteria causes infection occurs in respiratory tract, in wound or on the skin. It is spread by droplets or by contact to susceptible individuals that grow on mucous membranes producing toxin as Exotoxins which protein synthesis is responsible for the necroting and neurotoxin effects of diphtheria toxin. Diphtheria toxin is absorbed into the mucous membranes and

causes destruction of epithelium and a superficial inflammatory response. The necrotic epithelium becomes embedded in exuding fibrin and red and white cells, so that a grayish "pseudomembrane" is formed—commonly over the tonsils, pharynx, or larynx. Sore throat and fever dyspnea soon follow because of the obstruction caused by the membrane damage to the heart.

Diphtheria:

- Classified into pharyngeal or cutaneous diphtheria

- Pharyngeal diphtheria:

- Caused by toxin producing strain.
- Symptoms start with sore throat, fever, nausea, and croupy cough.
    - Characteristic dirty gray, fibrinous pseudo-membrane around tonsillar area, which bleeds if scraped.
    - The membrane may block airway and cause complete obstruction.
    - Neck edema and enlargement of cervical lymph nodes, causing swollen neck (bull neck).
    - Absorbed toxin may cause severe prostration, stupor, coma, or death.
- If untreated, diphtheria toxin may reach to distant organs and cause myocarditis, arrhythmias, neuritis, and acute tubular necrosis.

Cutaneous diphtheria:

Similar to chronic skin conditions such as eczema and psoriasis, usually on extremities; may look like punch out ulcers

❖ **- Risk factors:**

- Unvaccinated people

- Exposure to infected people
- Travel to endemic area
- Age group <15 years or >25 years

❖ **- Transmission:**

- Respiratory drops
- Contact of nasopharyngeal secretion
- Contact with skin lesion
- Fomites

Laboratory Diagnoses :-

- **Specimen:** Throat swab Gram stain: to demonstrate club-shape, gram positive, non spore forming, chines characters-like arrangement.
- PCR , -Diphtheria antibodies , - Elek's test for toxin.

- **Media for Culture:** Blood agar, potassium tellurite. Loeffler serum medium.

Treatment:

First choice: diphtheria antitoxin þ erythromycin or penicillin

- Second choice: diphtheria antitoxin

Prevention and control: Immunization (DPT Vaccine).

Listeria monocytogenes

Gram-positive bacilli, facultative intracellular, motile, with characteristic tumbling motility under light microscopy, Habitat of soil, decaying vegetables, animal feed, water, sewage, and human food.

❖ **Virulence factors:**

- Secret listeriolysin O toxin; it suppresses T-cell activation.

- Internalins: bacterial surface proteins, which help the bacteria in attaching to host cells

- Phosphatidylinositol-specific phospholipase helps the bacteria to escape from the host cell's vacuole.

❖ **Clinical significance:**

- Responsible for meningitis, bacteremia, encephalitis, gastroenteritis, and endocarditis.

- Infection in pregnancy may result in fetal loss, premature birth, or infected newborn (neonatal sepsis, meningitis, and death).

❖ **High risk group:**

➢ Neonates

➢ Older people >60 of years age

➢ Pregnant women

Mostly transmitted through food.

- High-risk contaminated food with Listeria are.

➢ Raw unpasteurized milk and anything made of it.

➢ Undercooked meat, deli meat, and poultry.

➢ Refrigerated smoked seafood.

➢ Unwashed salad, raw fruits, and vegetables.

- First-choice antibiotics:

- Ampicillin + gentamicin for meningitis and encephalitis
- Bacteremia / ampicillin

- Second-choice antibiotics:

Trimethoprim-sulfamethoxazole , Meropenem.

Acid-fast bacteria

(Mycobacteria)

The mycobacteria are rod-shaped, aerobic bacteria that do not form spores. Mycobacteria cannot be classified as either gram positive or gram negative. Although they do not stain readily, after being stained, they resist decolorization by acid or alcohol and are therefore called "acid-fast" bacilli. The **Ziehl-Neelsen technique** of staining is used for identification of acid-fast bacteria. *Mycobacterium tuberculosis* causes tuberculosis and is a very important pathogen of humans. *Mycobacterium leprae* causes leprosy. *Mycobacterium avium-intracellulare* and other nontuberculous (NTM) mycobacteria frequently infect patients with AIDS, are opportunistic pathogens in other immunocompromised persons, and occasionally cause disease in patients with normal immune systems. There are more than 200 *Mycobacterium* species, including many that are saprophytes.

Transmission :-

inhalation of airborne particles with bacteria

Risk factors:-

- Environment / infected untreated people disperse the bacteria in the surrounding, overcrowded, and poorly ventilated areas.
- People living in poor or underdeveloped areas.
- Health care providers who take care of patients with active disease.

❖ Sign and symptoms (three stages):-

- Primary infection
- Latent infection
- Active infection (Fig. 8.1).

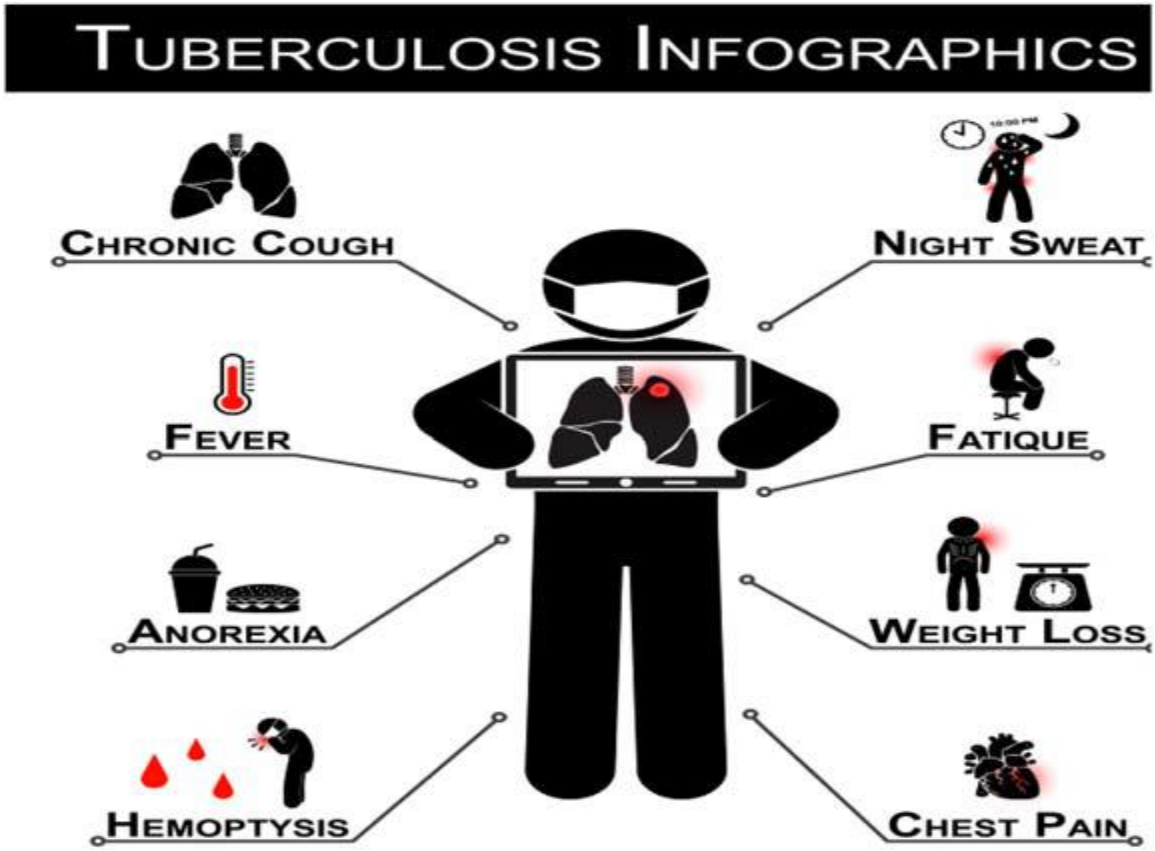


FIGURE 8.1 :Sign and symptoms of tuberculosis.

Treatment:

❖ Primary infection :

- Usually asymptomatic and noninfectious in adult immunocompetent people.
- In children and immunocompromised patients, primary infection may progress to acute illness.

- Inhale bacteria are engulfed by alveolar macrophages, where it survives by inhibiting the fusion of phagosome with lysosome. Bacteria multiply inside the macrophages and burst out, destroying the cell.
- Other inflammatory cells attract the area and wall off the bacteria called caseous granulomas or tubercles.
- Tubercles calcified in the middle or lower zone of lungs called Ghon focus.
- Ghon focus with perihilar lymph node called Ghon or Ranke complex.

❖ **Latent infection:**

- Organisms remain dormant and survive for years in these tubercles and reinfect the person as immunity decreases. Chance of reactivation is 10% in the lifetime of a common person.

❖ **Active infections:**

- Tuberculosis can affect any organ in the body. However, most common organ affected is lung.

A- Pulmonary tuberculosis : most common symptom is cough. Hemoptysis occurs in cavitary TB. Other symptoms are anorexia, fatigue, weight loss, night sweating, and low grades fever.

B- Extrapulmonary TB: (Pleural/pericardial TB) : results in fluid accumulation around the lungs and heart. - Lymph node TB : called scrofula. - Skeleton TB / Pott's disease. - CNS TB / subacute meningitis and granulomas.

- - Miliary TB / disseminated tubercules all over the body. Kidney, liver, lungs, and skin are all affected.

Evaluation for TB:

- Medical history , Physical examination , Mantoux tuberculin skin test (TST)
- Chest x-ray , Bacteriologic exam (smear and culture).

Laboratory Diagnosis of Mycobacterial Disease:

- Strongly consider TB in patients with smears containing acid-fast bacilli
- Use subsequent smear examinations to assess patient's infectiousness and response to treatment
- Culture : Used to confirm diagnosis of TB
- Culture all specimens, even if smear is negative, Eight week growth of *Mycobacterium tuberculosis* on Lowenstein-Jensen Agar.

❖ Treatment:

- First-line treatment : isoniazid + rifampin × 6-9 months+ pyrazinamide × 2 months + ethambutol × 2 months.
- For isoniazid-resistant strain : rifampin+ pyrazinamide + ethambutol + levofloxacin or moxifloxacin × 6-9 months.
- Multidrug-resistant strain (MDR).

Bedaquiline + pretomanid + linezolid × 26 weeks.

- For latent TB: Nine month of isoniazid once daily.

❖ Prevention

- A vaccine against *Mycobacterium tuberculosis* is available. It is called BCG (Bacillus of Calmette and Guerin, named after the two Frenchmen that developed it). BCG consists of a live attenuated strain derived from *Mycobacterium bovis*. This strain of Mycobacterium has remained a virulent for over 60 years.

Gram Negative Bacilli Bacteria
Family : Enterobacteriaceae and Vibrionaceae

Enterobacteriaceae

General characteristic

Enterobacteriaceae is a large family of Gram-negative rods (bacilli) bacteria ,whose natural habitat is the intestinal tract of human and animals. The family includes over 30 genera and more than 100 species (*Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Proteus* and others), referred to as "enteropathogenic bacteria" or enteric bacilli. Some enteric organisms e.g. *Escherichia coli*, are part of the normal flora and incidentally cause disease. Most of them are opportunistic pathogens infected human, general characteristics of it: Gram-negative rods, non-spore-forming facultative anaerobic, grow in simple media ferment glucose and produce acid, motile, have peritrichous flagella and capsulated.

Classification

Enterobacteriaceae have been classified on the basis of fermentation of lactose as shown below:

Table 9.1: Classification of Enterobacteriaceae.

Lactose fermenting	Nonlactose fermenting
Escherichia coli	*Proteus mirabilis*
Klebsiella pneumoniae	*Shigella dysenteriae*
Serratia	*Salmonella typhi* and nontyphi group
Enterobacter	*Yersinia enterocolitica*

1. Lactose fermenting

Escherichia coli :

- Most abundant gram-negative bacteria in the large intestine and part of normal flora
- Catalase positive, lactose fermenter, indole positive, capsulated bacteria with flagella
- Various serotypes (about 200 serotypes)
- Transmission is usually via fecal oral route, contaminated water, undercook hamburger or meat, and unpasteurized milk

Virulence factors:

1) Adhesins
2) Iron acquisition
3) Polysaccharide capsule
4) ToxinsLipopolysaccharide (LPS)

Antigenic structure

Escherichia coli can be classified on the bases of serotype :

- Somatic antigen present on the cell membrane is designated by a letter "O."
- Antigen present on the capsule is represented by a letter (K). Similarly, antigens located on fimbria are (F) and (H) antigens on bacterial flagella.
- Presence of these antigens makes the bacteria more virulent. For example, presence of K1 antigen is responsible for neonatal meningitis,

and the presence of O157:H7 is associated with hemorrhagic colitis, hemolytic uremic syndrome (HUS), and diarrhea.

E. coli can also be classified on the basis of phenotypes:

- Enterotoxigenic (ETEC) : toxin causes travelers diarrhea and watery diarrhea in infants. Does not cause blood in the stool.
- Enteroinvasive (EIEC) : inflammatory diarrhea , bloody diarrhea.
- Enterohemorrhagic or Shiga-like toxin-producing *E. coli* (EHEC or STEC) /serotype O157:H7 cause bloody diarrhea and Hemolytic Uremic Syndrome (HUS)
- Enteroaggregative : persistent diarrhea in AIDS patients.
- Enteropathogenic (EPEC) : watery diarrhea in infants and children.

Uropathogenic *E. coli* (UPEC): Cause urinary tract infection (UTI).

Clinical significance:

1) UTI / *E. coli* is the most common cause.
2) Gastroenteritis
3) Extraintestinal infections

- Cystitis (most common) , Bacteremia , Meningitis (especially in infants)
- Skin, pulmonary, and hepatobiliary infections
- **Choice of antibiotics:**

➢ First choice: third or fourth generation cephalosporin

➢ Second choice: ciprofloxacin or levofloxacin

- Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* / meropenem
- or ertapenem
- Resistance to meropenem / ceftazidime - avibactam Fosfomycin for cystitis or lower UTI

Klebsiella

Klebsiella pneumoniae is most common species. Klebsiella are non motile, gram negative, short and thick bacilli. They form large capsule which is responsible for mucoid colonies on media.

- **Virulence factors:**
- Capsule , Lipopolysaccharides (LPS)
- Fimbriae , Biofilm
- **Clinical significance:**
- wound sepsis , bacteraemia, meningitis
- UTI in a patient with urinary catheters
- Pneumonia in hospital and alcoholic patients, produce lung abscess
- **Choice of antibiotics:**
- First choice: third or fourth generation cephalosporin or fluoroquinolones.
- Second choice: piperacillin-tazobactam or aminoglycoside
- - ESBL-producing strain : meropenem or ceftazidime-avibactam

2. Nonlactose fermenting

These are not part of human intestinal flora but reside in the gut of animals such as chickens, eggs, pigs, turtles, snakes, fish, and other species.

Shigella:

General properties of Shigella:

Facultative anaerobic, nonmotile, nonlactose fermenting, urease and oxidase negative, and does not produce H2S. Spread via fecal oral route, contaminated food, and fomites. Human is the only natural reservoir, but the bacterium is not a part of normal flora of human intestine.

Four species of Shigella cause shigellosis, which is an acute infection of intestine. Species involved are:

- *Shigella dysenteriae*: Responsible for most severe form of shigellosis
- *Shigella flexneri*
- *Shigella boydii*
- *Shigella sonnei*

➢ **Virulence factors:**

- **Acid tolerance:** Survive at low PH
- **Effector proteins** :Shigella uses number of effector proteins that bind to host cells and lyse the phagosome and release the bacteria into cell cytoplasm.
- *S. dysenteriae* secrete toxin (Shiga toxin), which is very similar to EHEC and EIEC toxin.

Pathogenesis:

Shigella is the agent of bacterial dysentery shigellosis(stools containing blood and mucus). Shigella infection is typically by ingestion. Shigella species generally invade the epithelial lining of the colon, causing severe inflammation and death of the cells lining the colon. This inflammation results in the diarrhea and even dysentery that are the hallmarks of Shigella infection. Some strains of Shigella produce toxins which contribute to disease during infection. *S. flexneri* strains produce ShET1 and ShET2, which may contribute to diarrhea. *S. dysenteriae* strains produce Shiga toxin, which is hemolytic similar to the verotoxin produced by enterohemorrhagic *E. coli*.

Treatment:

Supportive therapy by fluid replacement . Antibiotics (First choice: Azithromycin, fluoroquinolone, third generation cephalosporin, and levofloxacin . Second choice: Trimethoprim-sulfamethoxazole. Avoid the use of antidiarrheal drugs / prolong the illness.

• Salmonella:

Gram-negative, motile, non-spore forming non-fermented lactose on MacConkey agar produce gas when fermenting glucose, produce H2S from thiosulfate. (TSI agar).

The bacterial genus Salmonella (S) consists of two species, *S. enterica* and *S. bongori*. We focus on *S. enterica* in the remaining section as it represents 99.5% of all Salmonella strains, while *S. bongori* is largely associated with reptiles . *S. enterica* is traditionally classified by serotype based on combinations of two

surface proteins, flagellar (H) and somatic (O) antigens. To date, more than 2600 serotypes have been reported. These serovars are also often grouped according to their clinical presentations in humans, typhoidal vs. non-typhoidal.

The former causes typhoid/enteric fever, a serious systemic condition that is often life-threatening, and includes *S. serovars Typhi* and *Paratyphi A,B,C*. The remaining serovars are considered non-typhoidal and represent the major cause of foodborne illness (gastroenteritis/diarrhea disease).

Many Salmonella serovars have a broad host range, infecting a wide variety of animals, including mammals, birds, reptiles, amphibians, fish, and insects, while others are very limited in their host range . Salmonella can also grow in plants and can survive in protozoa, soil, and water, extending its transmission routes. Chronic asymptomatic carriage of either type of Salmonella develops in some patients after initial infection, which is more often documented for typhoidal Salmonella. Broad-host-range, ubiquitous/generalist, Salmonella pathogens are generally non-typhoidal, but typhoidal serovars take only humans as the host .

a) *Salmonell typhi*

- Human is the only host and reservoir. Shed in stool or urine of asymptomatic carrier or patients with disease.
- Bacteria enter water or food through feces or urine due to unhygienic conditions and infect other people.
- From the gut, it reaches to the lymphatic system and bloodstream and causes typhoid fever.
- Endemic to Asia, Africa, Latin America, and the Caribbean.

➢ **Virulence factors:**

- Facultative intracellular
- Vi capsular polysaccharide antigen / protect the bacteria from being tagged
- with antibodies.
- Fimbriae
- Flagella

Typhoid fever

- Incubation period is about 1-2 weeks, and symptoms may last 4-6 weeks. It could be life threatening if not treated.

➢ Sign and symptoms:

- High-grade fever, headache, abdominal pain, constipation followed by diarrhea, and rose spots rash on the trunk.
- Splenomegaly, hepatomegaly, hepatitis, cholecystitis, and proteinuria may develop.
- Diarrhea with or without blood can develop.
- Other complications are / pneumonia, bacteremia, endocarditis, meningitis, and glomerulitis.

- **Diagnosis:**

➢ Clinical

➢ Culture

➢ Serology test / Widal test: Detects antibodies against the O and H antigens of *S. typhi*.

- **Treatment:**
- Mortality is high without treatment
- First choice: Ceftriaxone, azithromycin, or meropenem
- Second choice: Fluoroquinolone or chloramphenicol
- **Carrier state:**

➢ About 3% of patients who are not treated enter into chronic carrier state and harbor bacteria in their gallbladder. Some people with no clinical symptoms are silent carriers and shed the bacteria in the stool.

➢ Use fluoroquinolone or surgery to remove the gallbladder.

b) Nontyphoid salmonella

Nontyphoid salmonella causes various types of infections that involve various serotype.

- Bacteremia / prolongs fever, headache, malaise but usually no diarrhea.

Bacteremia may lead to focal infection of the bone (osteomyelitis), heart (endocarditis), lungs, joints, soft tissue, and genitourinary tract.

- Gastroenteritis / causes inflammatory diarrhea with mucus and blood, abdominal cramp, nausea and vomiting, headache, chill, and low-grade fever.

➢ **Diagnosis is clinical. Culture can be done.**

➢ **Choice of antibiotic:** Ciprofloxacin, azithromycin, and ceftriaxone are the first choice antibiotics.

➢ **Preventions** / washing hands with soap and water and preventing contamination of the food and water supply.

Other Gram Negative Bacilli

Pseudomonas aeruginosa

Pseudomonas is gram-negative, motile by monotrichous flagella, aerobic rods some of which produce water-soluble pigments. *P. aeruginosa* is widely distributed in nature and is commonly present in moist environments in hospitals. It can colonize normal humans, in whom it is a saprophyte. It causes disease in humans with abnormal host defenses. The strain of *P. aeruginosa* can produce four different types of pigments. Most commonly produced is blue – green pigment called pyocianin which diffused into the surrounding medium. Other Pseudomonas species do not produce pyocyanin. Many strains of *P aeruginosa* also produce the fluorescent pigment pyoverdin, which gives a greenish color to the agar .Some strains produce the dark red pigment pyorubin or the black pigment pyomelanin.

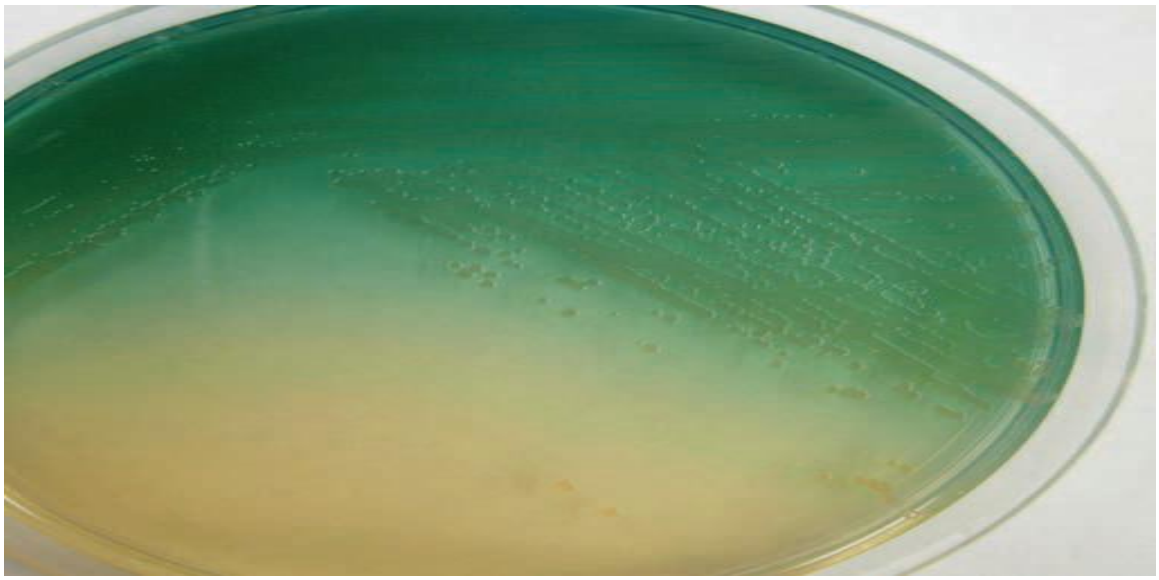


Figure 10–1 *Pseudomonas aeruginosa* on a Mueller-Hinton agar plate. The organism produces pyocyanin, which is blue, and pyoverdin, which is green. Together these pigments produce the blue green color that is seen in the agar around the pseudomonas growth.

Pathogenesis:

P .aeruginosa have many virulence factors: capsule, Pili, flagella, protease, Many strains of *P. aeruginosa* produce exotoxin A, which causes tissue necrosis and is lethal for animals when injected in purified form. The toxin blocks protein synthesis by a mechanism of action identical to that of diphtheria toxin. The bacterium attaches to and colonizes the mucous membranes or skin, invades locally, and produces systemic disease. These processes are promoted by the pili, enzymes, and toxins. Lipopolysaccharide plays a direct role in causing fever, shock, oliguria, leukocytosis and leukopenia, disseminated intravascular coagulation, and adult respiratory distress syndrome. *P. aeruginosa* may invade the bloodstream and result in fatal sepsis, also causes wound infections, Burn infections, UTIs, Bacteremia, Pneumonia and meningitis, Cystic fibrosis and other opportunistic infections.

Treatment and Prevention

Infections of Pseudomonas bacteria are often serious and very resistant to therapy. The penecillins can be used in combination with an aminoglycosides, usually gentamycin, amikacin or tobramycin. Ciprofloxacin has been found to be effective against *P. aeruginosa.* Other drugs active against *P. aeruginosa* include aztreonam; carbapenems such as imipenem or meropenem; and the fluoroquinolones, including ciprofloxacin.

Brucella (Brucellosis Bacilli)

Brucella organism cause brucellosis which is also known as malta fever, mediterranean fever, undulant fever or Rock fever. This genus is small, gram negative, non motile bacilli, usually coccobacillary in shape. This genus include three species are (*B. abortus*, *B. suis* and *B. melitensis*).

Pathogenicity:

Brucellosis is a zoonosis and man gets infection when he comes in close contact with animal or animal products. Drinking of unpasteurized milk is a major transmission route.

Clinical symptoms :

fever, weakness, malaise, headache and sweating. The fever may occur in cycles with febrile periods alternating with a febrile periods.

Diagnosis:

1- Direct demonstrate of organism by culture.

2- Indirect evidence in the form of specific antibody (serology) can be shown to establish the diagnosis.

Treatment:

Tetracycline is the most effective antibiotics against brucellosis. Streptomycin + tetracycline to prevent recurrence.

Helicobacter pylori

Curved, microaerophilic, gram-negative bacilli. It is urease, oxidase, and catalase positive. It is estimated that about 50% world's population is infected. Most people remain asymptomatic and are the silent carrier.

Transmission:

- Fecal oral or oral to oral
- Contaminated water supply
- Eating uncooked vegetables
- Use of inadequately disinfected medical devices such as endoscope
- Iatrogenic

Virulence factors:

- Flagella
- Adhesins
- Urease / convert urea into carbon dioxide and ammonia and neutralize the acid.
- Some strain carries a gene A or cagA that induce inflammatory response and cause gastritis
- CagA is also linked to the development of gastric cancers (MALT lymphoma and adenocarcinoma).
- Some *H. pylori* strain produces exotoxin (cytotoxin A), which destroys epithelial cells and exposes underlying mucosal layer to gastric acid.

Clinical significance:

- Cause duodenal ulcer, gastritis, gastric adenoma, and low-grade gastric adenoma.
- Symptoms depend on where in the stomach the infection is?
- Antral predominate causes increased gastrin production, which results in duodenal ulcer.
- Body predominate causes gastric atrophy and ↓acid production, which result in gastric ulcer and gastric adenocarcinoma.
- Chronic infection can lead to iron deficiency anemia.

Diagnosis:

- Urea breathe test / oral dose of urea with C 13 or C 14 is given. Bacteria metabolize urea and release label CO2 that is exhaled by the patient and is measured. Sensitivity and specificity are greater than 95%.
- The test is used to confirm the eradication of the bacteria after the therapy.
- Stool antigen test / sensitivity and specificity are the same. Remain positive for a while, even after the treatment.
- Endoscopy and biopsy.

Treatment:

Triple therapy / Proton pump inhibitors +amoxicillin or metronidazole + clarithromycin

Bordetella pertusis

B. pertusis is strictly aerobic, gram negative coccobacilli which require complex media for its growth. Whooping cough is an acute infectious disease caused by *B. pertusis*. It usually affects young children.

Virulence Factors:

Various virulence factors alone or together are responsible for producing clinical manifestigation. These factors include:

- Pilli
- Endotoxin and Exotoxin
- Tracheal cytotoxin
- Haemolysin
- Pertusis toxin
- Filamentous haemaglutinin

Pathogenecity:

the organism is transmitted to other by airborne route. This bacterium selectively attaches to the ciliated epithelial cells of the upper respiratory tract and growth is limited to the superficial tissues.

Stages of disease:

1- 1st stage Catarrhal stage: symptoms of upper respiratory tract which include sneezing, running nose and coughing for 1-2 weeks.

2- 2nd stage Episodes of uncontrollable cough. Each paroxysm consists of 5-20 rapid cough with the patient unable to breathe between the coughs.

3- Last stage Forced inspiratory breath cause the "whooping" sound, from which the disease has derived its name from this feature. Such prolonged coughing may lead to anoxia, expelling of mucus and vomiting.

Complications :

The major complications of the disease are bronchopneumonia and lung collapse. The pressure effects during coughing can lead to sub-conjunctival hemorrhage and cerebral hemorrhage leading to convulsions and coma.

Diagnosis :

By examination of samples from the URT during the early stage. Multiple samples (up to six) especially by the per nasal swab technique are recommended.

Culturing on suitable media and identification can be confirmed by the slide agglutination method.

Treatment:

Erythromycin is the drug of choice but is useful only if initiated within first 10 days of the disease. Alternatively, tetracycline or cotrimoxazole may be used.

Prevention:

Primary prevention can be achieved by the immunization of the infant with DPT vaccine.

Haemophilus

Gram-negative, coccobacillus, nonmotile, facultative anaerobic bacteria, which is catalase and oxidase-positive. Non-motile, non-spore forming, facultative anaerobic, *H. influenzae* can be classified into encapsulated and nonencapsulated strains. Encapsulated strain is associated with several serious infections.

Pathogenesis

cause meningitis, otitis media, sinusitis, pneumonia, bronchitis, conjunctivitis, arthritis, and bacteremia specialized capsulated. This is a group of small, pleomorphic bacteria that require enriched media, usually containing blood or its derivatives, for isolation. *H. influenzae* type b is an important human pathogen; *Haemophilus aegyptius* cause purulent conjunctivitis and bacteremia. *Haemophilus ducreyi* cause chancroid. Infect the people via skin abrasion.

Virulence factors:

- Polysaccharide capsule
- Biofilm
- Pili
- Endotoxin
- Protease / destroy immunoglobulin IgA

Clinical significance:

- Meningitis ,- Bacteremia ,- Pneumonia ,- Septic arthritis Conjunctivitis , Epiglottis , - Sinusitis

Treatment:

- First choice: Third- or fourth-generation cephalosporins, amoxicillin-clavulanate
- Second choice: Macrolide, levofloxacin, clarithromycin, or azithromycin

Bacteriodes

Gram negative, rod-shaped, nonspore-forming, obligate anaerobic bacteria. More than 30 species have been recognized. *Bacteroides fragilis* is the most common and virulent Bacteroides. Most common anaerobic bacteria of gut flora where they have mostly symbiotic relationship with the host. Bacteria assist in the breakdown of complex food molecules and release nutrients for the body use.

Virulence factors:

- Lipopolysaccharide capsule: Escapes phagocytosing the bacteria by immune cells.
- Enterotoxigenic: Secretes heat-labile zinc metalloprotease toxin that stimulates IL-8 and other cytokines that destroy intestinal cells and cause inflammatory diarrhea.

Clinical relevance:

- Appendicitis - Peritonitis
- Abdominal abscess - Diabetic foot ulcer
- Pneumonia and lung abscess - Brain abscess
- Cellulitis - Dental infection

Treatment:

- First choice: Metronidazole
- Second choice: Piperacillin-tazobactam, meropenem, amoxicillin-clavulanate.

Medical Mycology

Mycology is the branch of biology concerned with the study of fungi, including their genetic and biochemical properties, their taxonomy and their use to humans as a source for tinder, traditional medicine, food, as well as their dangers, such as toxicity or infection.

mycology was a branch of botany because, although fungi are evolutionarily more closely related to animals than to plants, this was not recognized until a few decades ago. Pioneer mycologists included Elias Magnus Fries, Christian Hendrik Persoon, Anton de Bary, Elizabeth Eaton Morse, Lewis David von Schweinitz. Fungi are fundamental for life on earth in their roles as symbionts, e.g. in the form of mycorrhizae, insect symbionts, and lichens. Many fungi are able to break down complex organic biomolecules such as lignin, the more durable component of wood, and pollutants such as xenobiotics, petroleum, and polycyclic aromatic hydrocarbons. By decomposing these molecules, fungi play a critical role in the global carbon cycle.

Fungi are economically and socially important, as some cause diseases of animals (including humans) and of plants.

Table 11.1:Differences between Bacteria and fungi

Basis for Comparison	Bacteria	Fungi
Definition	Bacteria are single-celled microscopic organisms that are characterized by the presence of incipient nucleus and few membrane-less cell organelles.	Fungi, singular fungus, are eukaryotes that are characterized by the presence of chitin in the cell wall.
Cell Type	All bacteria are prokaryotes.	All fungi are eukaryotes.
No. of cells	Bacteria are unicellular organisms with simpler cellular structure.	Most fungi are multicellular with complex cellular structures. Some fungi like yeast might be unicellular.
Size	The size of bacteria ranges from 0.5 to 5 µm.	The size of the fungi ranges from 2 to 10

		µm.
Cell wall	The cell wall of bacteria is made up of peptidoglycan under which a cell membrane is present.	The cell wall of fungi is made up of chitin.
Morphology	Bacteria are found to have three distinct shapes , round (cocci), spiral (Spirilla), and rod-shaped (bacillus).	Fungi are found to have varying shapes, but most of them are spotted in the form of a thread-like structure called hyphae.
pH	Bacteria grow best in the neutral environment of pH range 6.5-7.	Fungi mostly prefer a slightly acidic environment with pH value 4-6.
Mobility	Some bacteria are motile with flagella.	Fungi are immobile organisms.
Cell organelles	Bacteria have few membrane-less organelles.	Fungi contain several membrane-bound organelles.
Ribosomes	Bacteria like all prokaryotes contain 70S ribosomes. 70S ribosomes consist of 50S and 30S subunits.	Fungi, like all eukaryotes, contain 80S ribosomes. The 80S ribosome is composed of two subunits 60S and 40S.
Reproduction	Bacteria reproduce by an asexual method like binary fission.	Fungi reproduce through both asexual and sexual methods.
Nutrition	Bacteria can be autotrophs or heterotrophs.	Fungi are mostly heterotrophs that feed on dead and decaying matter.
Source of energy	Bacteria derive their energy from inorganic matter or organic matter like sugar, protein, or fat.	Fungi obtain their energy from pre-existing organic matter.
Respiration	Bacteria perform aerobic and anaerobic respiration.	Most fungi like yeast perform ethanol fermentation or anaerobic respiration.
Cytoskeleton	Bacteria do not have cytoskeletons like microtubules or microfilaments.	Fungi have both microtubules and microfilaments.
Cell cycle	Bacteria have shorter cell cycles ranging from 20 to 60 minutes.	Fungi have longer cell cycles ranging from 12 to 24 hours.

General differences between fungi and other eukaryotes

Differences between fungi and plants:

1- plants make their own food by photosynthesis, while fungi are unable to make their own, but are saprophytic or parasitic

2- fungi do not have chlorophyll as in plants

3- fungi reproduce by spores, while plants reproduce by seeds and pollen

4- plants have roots to fix them in the soil, but in fungi there are no complex root systems or leaves

5- in an ecosystem, plants are productive while fungi are decomposers

6- the cell wall in plants is made of cellulose while in fungi it is made of chitin

7- plants are multicellular, while fungi may be unicellular or multicellular

Differences between fungi and animals:

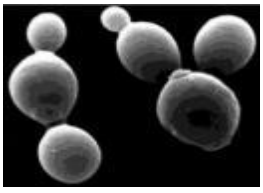
In fungi the nutrition is external, while in animal is mostly is internal

Animals produce cholesterol, while fungi produce ergosterol

Morphological classification of fungi

A. Yeasts
Unicellular, Nucleated rounded fungi

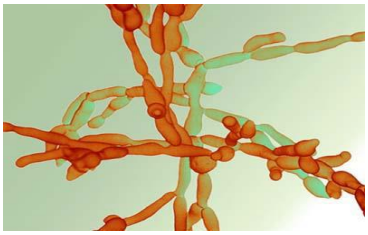


•Reproduce by budding
•Colony on solid media are usually white to beige and appear much like bacterial colonies
•Some genera produce mucoid colonies
•Yeast are used in the preparation in the variety of foods

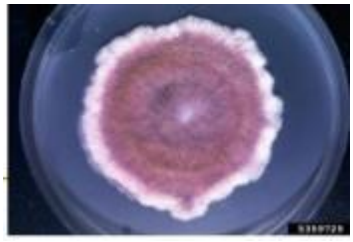
B. Yeast like fungi

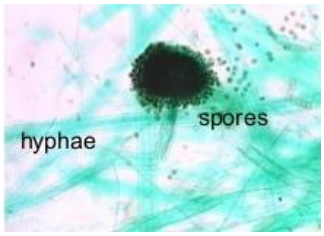
•Grow partly as yeasts and partly as elongated cells resembling hyphae which are called pseudo hyphae
•e.g. *Candida albicans*



C. Molds

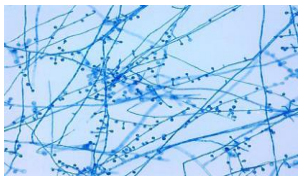
Multicellular hyphae
•Produce conidia (conidiospores)
•Colonies on solid agar are downy, fluffy, cottony
•Most mold colonies are pigmented and are useful in identification
•ex: *Penicillium* and *Cephalosporium*





D. Dimorphic fungi

•Occur in 2 forms:
Molds (Filaments) at 25 ^{0}C (in soil), and Yeasts at 37 ^{0}C (in host tissue)
•Most fungi causing systemic infections are dimorphic:
Histoplasma capsulatum
Blastomyces dermatidis



E. Fleshy fungi

A mushroom or toadstool is the fleshy, spore-bearing body of a fungus, typically produced over ground, on soil, or on its food source . it belong to the phylum **Basidiomycota**

Fungi reproduction (sexual and asexual)

Asexual reproduction: also called somatic or vegetative.

- Vegetative fragmentation method
- Transverse fission
- Budding
- Sclerotia
- spores

sexual reproduction: it done in three consecutive steps

1-plasmogamy
2-karyogamy
3-meiosis

Types of sexual reproductive

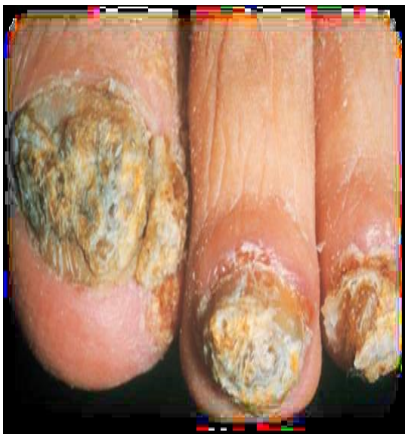
1-isogamy
2-heterogamy
3-gametes lose their cilia and remain within the gametophyte. The gametophytes are distinguished into large and small gametophytes
4- mold fungi have a special method by the union of parts of fungal hyphae .

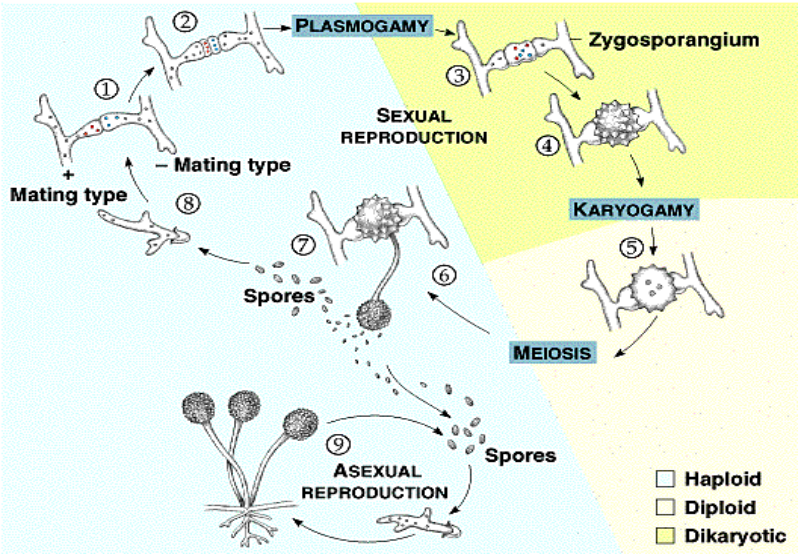
Classification of fungal diseases

A. Superficial mycosis
(The skin, hair, nail and mucous membranes)

1.Dermatophytosis (Ringworm) Form

Is a complex of diseases affecting the outermost keratinized tissues of hair, nail and parts of the skin Caused by dermatophytes mold fungi



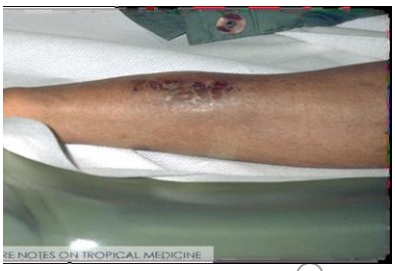


2.Yeast infections

Affect the skin, nail and the mucous membrane of the mouth and vagina
Usually caused by *Candida* species

B. Subcutaneous mycosis

Subcutaneous types include : sporotrichosis, mycetoma and chromoblastomycosis, which generally affect deeper tissues in the epidermis and the dermis**.** There is usually a rash with superficial infection. Fungal infection within the skin or under the skin may present with a lump and skin changes.



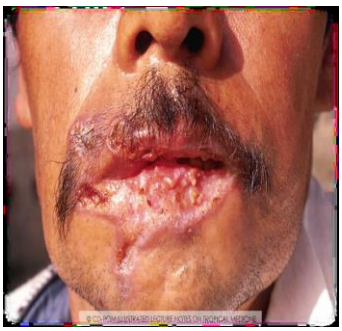
C. Systemic mycosis

Are more serious fungal infections and include : cryptococcosis (yeast Cryptococcus) , histoplasmosis (Histoplasma), pneumocystis pneumonia , aspergillosis (Aspergillus) and mucormycosis. Initially as a pulmonary infection through inhalation of air-borne spores ,then may disseminated to other organ.

–Caused by:

•Primary pathogens

•Opportunistic pathogens



Black fungus disease (Mucormycosis) is a severe invasive fungal infection caused by fungal species of mucorales typically seen in immunocompromised individuals. There has been an increased incidence of this fungal infection in patients suffering from **COVID 19 disease.** It most commonly infects the nose, sinuses, eye, and brain resulting in a runny nose, one-sided facial swelling and pain, headache, fever, blurred vision, bulging or displacement of the eye (proptosis), and tissue death. Other forms of disease may infect the lungs, stomach and intestines, and skin. It is spread by spores of molds of the order Mucorales, through inhalation, contaminated food, or contamination of open wounds. These fungi are common in soils, decomposing organic matter (such as rotting fruit and vegetables), and animal manure, but usually do not affect people. It is not transmitted between people. Risk factors include diabetes with persistently high blood sugar levels or

diabetic ketoacidosis, low white cells, cancer, organ transplant, iron overload, kidney problems, long-term steroids or use of immunosuppressant, and in HIV/AIDS.

* Mycetismus may caused by eating poisonous fungi as mushrooms (ex: Amanita spp.)

* Mycotoxicosis by eating moldy food (mycotoxins : Aflatoxin from *Aspergillus flavus*)

Laboratory diagnosis

Types of specimens:

***** skin, hair, & nails, ears, and mucosae

*****sputum, exudates, urine, blood, CSF, tissue biopsies

Common fungal primary recovery culture media are:

1.SAB agar (sabouraud dextrose agar)

2.Brain-Heart infusion agar

3.Potato flacke agar: These media used for primary recovery of saprophytic & dimorphic fungi.

4.Mycosel: these media used for identification of dermatophytes

Direct examination

1-potassium hydroxide (10-20%)

The most rapid method for direct examination of infected material, a small piece of infected position should be mixed with KOH (10-20% OR 30%), gently heating

the slide, then cover it with cover slip. The heating will increase the rate of clearing & the fungus should be more observed. The benefit of using KOH is to decomposition of natural skin.

2-cultivation of fungi

Place a small amount of hyphae or spores (or both) on the center of the agar medium in a Petri dish by using inoculating needle .

Temperature requirements : majority of fungi (37˚C), superficial mycosis(30˚C), Dimorphic fungi(25-37˚C).

Incubating time : usually cultures are obtained in 7-10 days, *Candida* & *Aspergillus* (24-72 hours) and *Cryptococcus* (up to 6 weeks).

Observe the development of colony over a period of incubation, noting **rate of growth**, **texture**, **pigmentation** on the surface and reverse side, as well as **folds or ridges** on the surface.

Other tests:

•Histology •Serology –ELISA –Immunodiffusion –PCR

Antifungal therapy

Fungal infections are hard to treat and can take a while to completely disappear. Treatment usually involves antifungal medications **fluconazole, ketoconazole, terbinafine and itraconazole** are oral agents reserved for extensive or severe infection which topical antifungal agents are inappropriate or ineffective, because of high cost, potential side effects and drug interactions. Topical antifungals (creams, liquids or spray) are used to treat fungal infections of the skin and nails . they include **clotrimazole, econazole, ketoconazole, miconazole, terbinafine, and amorolfine**. They come in various different brand names.

Glossary

Antibody: A protein produced mainly by plasma cells that is used by the immune system to neutralize pathogens such as pathogenic bacteria and viruses.

Antigen: A toxin or other foreign substance which induces an immune response in the body, especially the production of antibodies.

Autoimmunity: Immune responses of an organism against its own healthy cells and tissues.

Bacteremia: Presence of bacteria in the blood.

B - lactamase: An enzyme that cleaves the B- lactam ring of penicillin and related the antibiotic destroying their antibacterial activity.

Carrier: A person who harbors the microorganism without showing any signs or symptoms of the disease.

Cellulitis: Inflammation of subcutaneous connective tissue.

Culture media : It is liquid or solid medium designed to support the growth of microorganisms**.**

Dysentery: Presence of blood and mucus in the faces.

Erysipelas: Bacterial infection which is characterized by large raised red patches on the skin**.**

Halophilic: Organism that needs high salt concentrations for growth.

Meningitis: Inflammation of meninges of brain.

Neurotoxins: Poison which acts on the nervous system.

Reservoir: A place or a person where the infectious agent can live and multiply.

Septicemia: Invasion of the blood stream by virulent microorganisms along with their toxins.

Zoonosis: Disease which can be transmitted to humans from animals.

References

Al-Kobaisi, M. F. (2007). Jawetz, Melnick & Adelberg's Medical Microbiology 24th Edition. *Sultan Qaboos University Medical Journal*, *7*(3), 273-275.

Carroll, K. C., Hobden, J. A., Miller, S., Morse, S. A., Mietzner, T. A., Detrick, B., ... & Jawetz, M. (2019). *Adelberg's Medical Microbiology, 27e*. New York, NY:: McGraw-Hill Education.

Goering, R., Dockrell, H. M., Zuckerman, M., & Chiodini, P. L. (2023). *Mims' Medical Microbiology E-Book: Mims' Medical Microbiology E-Book*. Elsevier Health Sciences.

Murray, P. R., Rosenthal, K. S., & Pfaller, M. A. (2015). *Medical microbiology*. Elsevier Health Sciences.

Parija, S. C. (2023). *Textbook of microbiology and immunology*. Berlin, Heidelberg, Germany: Springer.