PAPER –BTY 103 MICROBIOLGY

Unit-I

Bacteria Cell Structure

They are as unrelated to human beings as living things can be, but bacteria are essential to human life and life on planet Earth. Although they are notorious for their role in causing human diseases, from tooth decay to the Black Plague, there are beneficial species that are essential to good health.

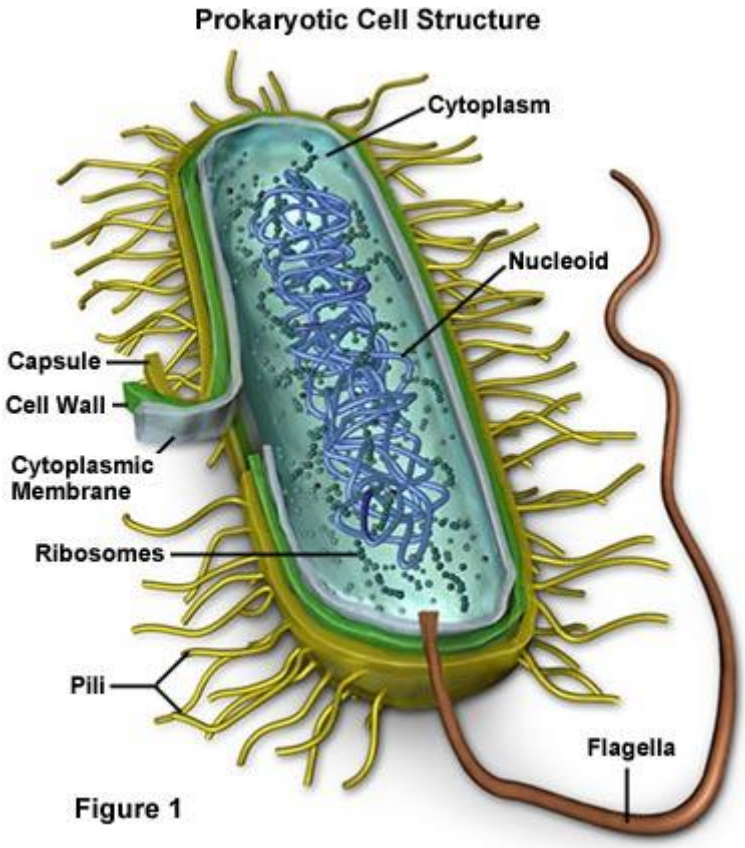


Figure 1

For example, one species that lives symbiotically in the large intestine manufactures vitamin K, an essential blood clotting factor. Other species are beneficial indirectly. Bacteria give yogurt its tangy flavor and sourdough bread its sour taste. They make it possible for ruminant animals (cows, sheep, goats) to digest plant cellulose and for some plants, (soybean, peas, alfalfa) to convert nitrogen to a more usable form.

Bacteria are prokaryotes, lacking well-defined nuclei and membrane-bound organelles, and with chromosomes composed of a single closed DNA circle. They come in many shapes and sizes, from minute

spheres, cylinders and spiral threads, to flagellated rods, and filamentous chains. They are found practically everywhere on Earth and live in some of the most unusual and seemingly inhospitable places.

Evidence shows that bacteria were in existence as long as 3.5 billion years ago, making them one of the oldest living organisms on the Earth. Even older than the bacteria are the archeans (also called archaebacteria) tiny prokaryotic organisms that live only in extreme environments: boiling water, super-salty pools, sulfur-spewing volcanic vents, acidic water, and deep in the Antarctic ice. Many scientists now believe that the archaea and bacteria developed separately from a common ancestor nearly four billion years ago. Millions of years later, the ancestors of today's eukaryotes split off from the archaea. Despite the superficial resemblance to bacteria, biochemically and genetically, the archea are as different from bacteria as bacteria are from humans.

In the late 1600s, Antoni van Leeuwenhoek became the first to study bacteria under the microscope. During the nineteenth century, the French scientist Louis Pasteur and the German physician Robert Koch demonstrated the role of bacteria as pathogens (causing disease). The twentieth century saw numerous advances in bacteriology, indicating their diversity, ancient lineage, and general importance. Most notably, a number of scientists around the world made contributions to the field of microbial ecology, showing that bacteria were essential to food webs and for the overall health of the Earth's ecosystems. The discovery that some bacteria produced compounds lethal to other bacteria led to the development of antibiotics, which revolutionized the field of medicine.

There are two different ways of grouping bacteria. They can be divided into three types based on their response to gaseous oxygen. Aerobic bacteria require oxygen for their health and existence and will die without it. Anerobic bacteria can't tolerate gaseous oxygen at all and die when exposed to it. Facultative aneraobes prefer oxygen, but can live without it.

The second way of grouping them is by how they obtain their energy. Bacteria that have to consume and break down complex organic compounds are heterotrophs. This includes species that are found in decaying material as well as those that utilize fermentation or respiration. Bacteria that create their own energy, fueled by light or through chemical reactions, are autotrophs.

- **Capsule** - Some species of bacteria have a third protective covering, a capsule made up of polysaccharides (complex carbohydrates). Capsules play a number of roles, but the most important are to keep the bacterium from drying out and to protect it from phagocytosis (engulfing) by larger microorganisms. The capsule is a major virulence factor in the major disease-causing bacteria, such

as ***Escherichia coli*** and ***Streptococcus pneumoniae***. Nonencapsulated mutants of these organisms are avirulent, i.e. they don't cause disease.

- **Cell Envelope** - The cell envelope is made up of two to three layers: the interior cytoplasmic membrane, the cell wall, and -- in some species of bacteria -- an outer capsule.
- **Cell Wall** - Each bacterium is enclosed by a rigid cell wall composed of peptidoglycan, a protein-sugar (polysaccharide) molecule. The wall gives the cell its shape and surrounds the cytoplasmic membrane, protecting it from the environment. It also helps to anchor appendages like the pili and flagella, which originate in the cytoplasm membrane and protrude through the wall to the outside. The strength of the wall is responsible for keeping the cell from bursting when there are large differences in osmotic pressure between the cytoplasm and the environment.

  Cell wall composition varies widely amongst bacteria and is one of the most important factors in bacterial species analysis and differentiation. For example, a relatively thick, meshlike structure that makes it possible to distinguish two basic types of bacteria. A technique devised by Danish physician Hans Christian Gram in 1884, uses a staining and washing technique to differentiate between the two forms. When exposed to a gram stain, gram-positive bacteria retain the purple color of the stain because the structure of their cell walls traps the dye. In gram-negative bacteria, the cell wall is thin and releases the dye readily when washed with an alcohol or acetone solution.

- **Cytoplasm** - The cytoplasm, or protoplasm, of bacterial cells is where the functions for cell growth, metabolism, and replication are carried out. It is a gel-like matrix composed of water, enzymes, nutrients, wastes, and gases and contains cell structures such as ribosomes, a chromosome, and plasmids. The cell envelope encases the cytoplasm and all its components. Unlike the eukaryotic (true) cells, bacteria do not have a membrane enclosed nucleus. The chromosome, a single, continuous strand of DNA, is localized, but not contained, in a region of the cell called the nucleoid. All the other cellular components are scattered throughout the cytoplasm.

  One of those components, plasmids, are small, extrachromosomal genetic structures carried by many strains of bacteria. Like the chromosome, plasmids are made of a circular piece of DNA. Unlike the chromosome, they are not involved in reproduction. Only the chromosome has the genetic instructions for initiating and carrying out cell division, or binary fission, the primary means of reproduction in bacteria. Plasmids replicate independently of the chromosome and, while not essential for survival, appear to give bacteria a selective advantage.

  Plasmids are passed on to other bacteria through two means. For most plasmid types, copies in the cytoplasm are passed on to daughter cells during binary fission. Other types of plasmids, however,

form a tubelike structure at the surface called a pilus that passes copies of the plasmid to other bacteria during conjugation, a process by which bacteria exchange genetic information. Plasmids have been shown to be instrumental in the transmission of special properties, such as antibiotic drug resistance, resistance to heavy metals, and virulence factors necessary for infection of animal or plant hosts. The ability to insert specific genes into plasmids have made them extremely useful tools in the fields of molecular biology and genetics, specifically in the area of genetic engineering.

- **Cytoplasmic Membrane** - A layer of phospholipids and proteins, called the cytoplasmic membrane, encloses the interior of the bacterium, regulating the flow of materials in and out of the cell. This is a structural trait bacteria share with all other living cells; a barrier that allows them to selectively interact with their environment. Membranes are highly organized and asymmetric having two sides, each side with a different surface and different functions. Membranes are also dynamic, constantly adapting to different conditions.
- **Flagella** - Flagella (singular, flagellum) are hairlike structures that provide a means of locomotion for those bacteria that have them. They can be found at either or both ends of a bacterium or all over its surface. The flagella beat in a propeller-like motion to help the bacterium move toward nutrients; away from toxic chemicals; or, in the case of the photosynthetic cyanobacteria; toward the light.
- **Nucleoid** - The nucleoid is a region of cytoplasm where the chromosomal DNA is located. It is not a membrane bound nucleus, but simply an area of the cytoplasm where the strands of DNA are found. Most bacteria have a single, circular chromosome that is responsible for replication, although a few species do have two or more. Smaller circular auxiliary DNA strands, called plasmids, are also found in the cytoplasm.
- **Pili** - Many species of bacteria have pili (singular, pilus), small hairlike projections emerging from the outside cell surface. These outgrowths assist the bacteria in attaching to other cells and surfaces, such as teeth, intestines, and rocks. Without pili, many disease-causing bacteria lose their ability to infect because they're unable to attach to host tissue. Specialized pili are used for conjugation, during which two bacteria exchange fragments of plasmid DNA.
- Ribosomes - Ribosomes are microscopic "factories" found in all cells, including bacteria. They translate the genetic code from the molecular language of nucleic acid to that of amino acids—the building blocks of proteins. Proteins are the molecules that perform all the functions of cells and living organisms. Bacterial ribosomes are similar to those of eukaryotes, but are smaller and have a slightly different composition and molecular structure. Bacterial ribosomes are never bound to other organelles as they sometimes are (bound to the endoplasmic reticulum) in eukaryotes, but are free-standing structures distributed throughout the cytoplasm. There are sufficient differences between bacterial ribosomes and eukaryotic ribosomes that some antibiotics will inhibit the functioning of

bacterial ribosomes, but not a eukaryote's, thus killing bacteria but not the eukaryotic organisms they are infecting.

Bacteria are a large group of minute, unicellular, microscopic organisms, which have been classified as prokaryotic cells, as they lack a true nucleus. These microscopic organisms comprise a simple physical structure, including cell wall, capsule, DNA, pili, flagellum, cytoplasm and ribosomes.

Bacteria can be gram-positive or gram-negative depending upon the staining methods. Let us have a detailed look at the difference between the two types of bacteria.

Gram Staining

This technique was proposed by Christian Gram to distinguish the two types of bacteria based on the difference in their cell wall structures. The gram-positive bacteria retain the crystal violet dye, which is because of their thick layer of peptidoglycan in the cell wall.

This process distinguishes bacteria by identifying peptidoglycan that is found in the cell wall of the gram-positive bacteria. A very small layer of peptidoglycan is dissolved in gram-negative bacteria when alcohol is added.

Gram-Positive and Gram-Negative Bacteria – Overview

The gram-positive bacteria retain the crystal violet colour and stain purple whereas the gram-negative bacteria lose crystal violet and stain red. Thus, the two types of bacteria are distinguished by gram staining.

Gram-negative bacteria are more resistant to antibodies because their cell wall is impenetrable.

Gram-positive and gram-negative bacteria are classified based on their ability to hold the gram stain. The gram-negative bacteria are stained by a counterstain such as safranin, and they are de-stained because of the alcohol wash. Hence under a microscope, they are noticeably pink in colour. Gram-positive bacteria, on the other hand, retains the gram stain and show a visible violet colour upon the application of mordant (iodine) and ethanol (alcohol).

Gram-positive bacteria constitute a cell wall, which is mainly composed of multiple layers of peptidoglycan that forms a rigid and thick structure. Its cell wall additionally has teichoic acids and phosphate. The teichoic acids present in the gram-positive bacteria are of two types – the lipoteichoic acid and the teichoic wall acid.

In gram-negative bacteria, the cell wall is made up of an outer membrane and several layers of peptidoglycan. The outer membrane is composed of lipoproteins, phospholipids, and LPS. The

peptidoglycan stays intact to lipoproteins of the outer membrane that is located in the fluid-like periplasm between the plasma membrane and the outer membrane. The periplasm is contained with proteins and degrading enzymes which assist in transporting molecules.

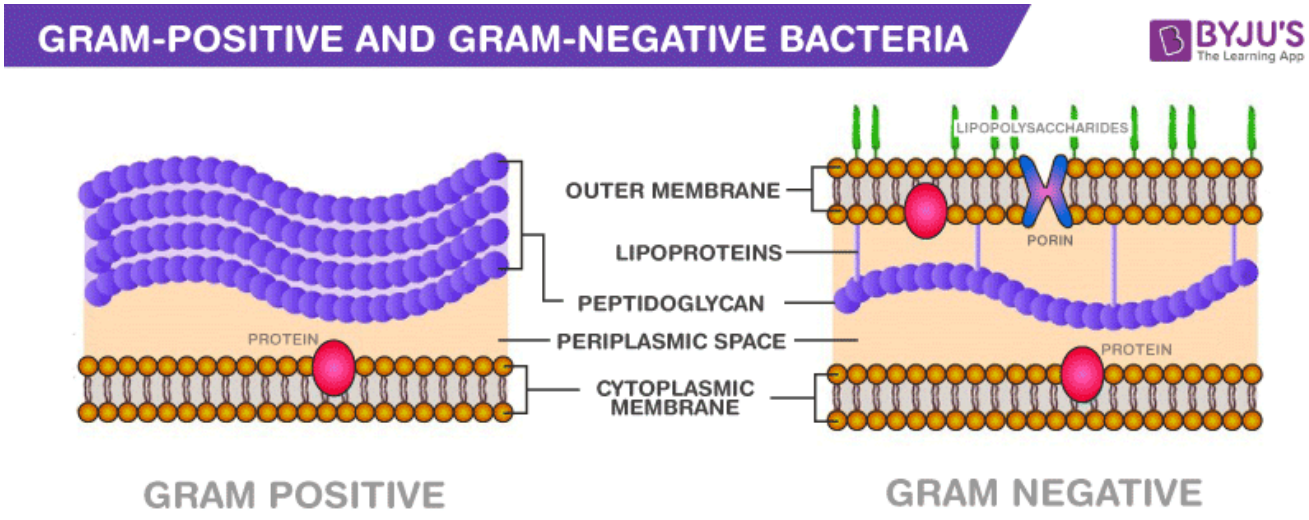
The cell walls of the gram-negative bacteria, unlike the gram-positive, lacks the teichoic acid. Due to the presence of porins, the outer membrane is permeable to nutrition, water, food, iron, etc.

Difference between Gram-Positive and Gram-Negative Bacteria – Key Points

- The cell wall of gram-positive bacteria is composed of thick layers peptidoglycan.
- The cell wall of gram-negative bacteria is composed of thin layers of peptidoglycan.
- In the gram staining procedure, gram-positive cells retain the purple coloured stain.
- In the gram staining procedure, gram-negative cells do not retain the purple coloured stain.
- Gram-positive bacteria produce exotoxins.
- Gram-negative bacteria produce endotoxins.

Difference between Gram-Positive and Gram-Negative Bacteria

Following are the important differences between gram-positive and gram-negative bacteria:



Difference between Gram-Positive and Gram-Negative Bacteria

Gram-Positive bacteria	Gram-Negative bacteria

Cell Wall	
A single-layered, smooth cell wall	A double-layered, wavy cell-wall
Cell Wall thickness	
The thickness of the cell wall is 20 to 80 nanometres	The thickness of the cell wall is 8 to 10 nanometres
Peptidoglycan Layer	
It is a thick layer/ also can be multilayered	It is a thin layer/ often single-layered.
Teichoic acids	
Presence of teichoic acids	Absence of teichoic acids
Outer membrane	
The outer membrane is absent	The outer membrane is present (mostly)
Porins	
Absent	Occurs in Outer Membrane
Mesosome	
It is more prominent.	It is less prominent.
Morphology	
Cocci or spore-forming rods	Non-spore forming rods.
Flagella Structure	
Two rings in basal body	Four rings in basal body

Lipid content	
Very low	20 to 30%
Lipopolysaccharide	
Absent	Present
Toxin Produced	
Exotoxins	Endotoxins or Exotoxins
Resistance to Antibiotic	
More susceptible	More resistant
Examples	
Staphylococcus, Streptococcus, etc.	Escherichia, Salmonella, etc.
Gram Staining	
These bacteria retain the crystal violet colour even after they are washed with acetone or alcohol and appear as purple-coloured when examined under the microscope after gram staining.	These bacteria do not retain the stain colour even after they are washed with acetone or alcohol and appear as pink-coloured when examined under the microscope after gram staining

UNIT-II

Sterilization- Physical and chemical methods

Microorganisms play an important role in causing infection and contamination. Therefore, Sterilization is an important technique in microbiology which helps to remove or destroy microorganisms from materials or surfaces.

Sterilization

It is the process by which an article, surface or medium is made free of all microorganisms either in vegetative or spore form.

Methods of Sterilization

Physical Methods

Sunlight

Sunlight has an active germicidal effect due to the presence of ultraviolet rays. It is a natural procedure of sterilization which reduces the number of microorganisms in water tanks, lakes, etc.

Heat

Heat is a mostly used method of sterilization. Moreover, it is a highly effective and most reliable process. There are two major methods of using heat in sterilization which are dry heat and moist heat. The principle behind both of these methods is similar. Dry heat induces the denaturation of protein, oxidative damage and toxic effect due to the high level of electrolytes. Moreover, the dry heat can also damage the DNA of the microorganism. As a result, the microorganism got killed. Moist Heat kills the microorganisms by denaturation and coagulation of proteins. There are several factors that can influence the heat killing procedure. Such as

- Temperature and Duration: The duration and temperature are inversely connected to each other. Therefore, in the case of the long duration of heat provided for sterilization, the temperature will be reduced while in case of high temperature the duration will be reduced.
- Characteristic of the Microorganism: Microorganisms can be present in both vegetative and spore form. Spore forms are generally heat resistant. Therefore, the sterilization process will vary on the basis of the characteristic of the microorganism.
- Type of Material: Organic substances often provide protection to the vegetative and spore form of microorganisms which reduces the lethal property of heat. Apart from that, the materials containing the substances are also needed to be heat stable for proper sterilization.

Dry Heat: Procedures

Red Heat

Inoculation loops, wires, forceps tips, needles are needed to be sterilized to inhibit microbial contamination. These instruments are held in the flame of a Bunsen burner until they become red hot.

Flaming

Glass slides, scalpels, and mouths of culture tubes or conical flasks are passed through Bunsen flame without allowing them to become red hot.

Incineration

This procedure is used to reduce the infective material into ashes by burning. The incinerator is used for the process. Soiled dressings, animal carcasses, bedding, and pathological materials are dealt with this method.

Hot Air Oven

It is a widely used method of sterilization by dry heat. The heat inside the oven is maintained by electricity and a fan fitted inside it provides the adequate distribution of hot air inside the chamber. A thermostat is also connected which maintains the temperature inside the chamber. 160^0C for two hours is required for sterilization. There are also some alternative temperatures and holding time which include 170^0C for 1 hour and 180^0C for 30 minutes.

Uses: Sterilization of

- Glasswares like glass syringes, Petri dishes, flasks, pipettes, and test tubes.
- Surgical instruments like scalpels, scissors, forceps, etc.
- Chemicals such as liquid, paraffin, fats, sulphonamides powders etc.

Sterilization control

- The spores of Bacillus subtilis subsp. Niger (NCTC 10075 or ATCC 9372) are kept inside the oven. These spores should be destroyed if the sterilization is proper.
- Thermocouples may also be used.
- Browne's tube with a green spot is available. After proper sterilization, a green color is produced (after two years at 160^0C).

Moist Heat Sterilization: Procedure

Temperature below 100^0C

Pasteurization

There are two different types of pasteurization methods that are used for sterilization of milk, Holder method (63^oC for 30 minutes) and flash method (72^0C for 20 seconds followed by cooling quickly to 13^0C). This method is effective against all non-sporing pathogens such as mycobacteria, *Salmonella*, etc. except *Coxiella burnetii* which survives the holder method due to heat resistant characteristics.

Inspissation

Media like Lowenstein- Jensen's and Loeffler's serum are required to sterile at 80-85^{0}C for 30 minutes daily on three consecutive days. This process is known as inspissation and the instrument used is called inspissator.

Vaccine bath

It is used for sterilization of bacterial vaccines at 60^{0}C for one hour. Serum or other body fluids can be sterilized by heating in a water bath at 56^{0}C for several successive days.

Low-temperature steam formaldehyde sterilization (LTSF)

This method is applicable for materials that cannot withstand 100^{0}C temperature. In this method, steam at subatmospheric pressure at 75^{0}C with formaldehyde vapor is used. *Bacillus stearothermophilus* plays an important role as a biological control to test the efficacy of the test.

At a temperature of 100^{0}C

Boiling

It is an effective method that can kill vegetative cells. Boiling for 10-30 minutes can kill most of the vegetative cells; however, many spores can withstand this temperature. Boiling can be employed when adequate methods are not available to sterilize glass syringes, rubber stopper, etc.

Tyndallisation

In this case, steam at 100^{0}C for successive 3 days is used. It is also known as intermittent sterilization. In this case, the first exposure kills the vegetative forms, and in the intervals between the heating and remaining spores germinates into vegetative forms which are killed on subsequent heating. This process is applied for sterilization of egg, serum or sugar-containing media which can be damaged due to exposure in high temperature for a longer period.

Steam sterilizer

Koch's and Arnold's steam sterilization is usually used for media which can easily decompose due to the high temperature in the autoclave. Those media are kept on a perforated tray and steam at 100^{0}C and at atmospheric pressure passes through the media for 90 minutes. It is an effective method to kill vegetative cells.

Temperature above 100^{0}C (under pressure)

Autoclave

Steam above 100^{0}C or saturated steam has a better killing capacity than dry heat. Bacterial proteins coagulate rapidly at moist heat. Saturate steam has the ability to penetrate any porous material. When steam comes into contact with the cooler surface it condenses into water and releases its latent heat to the surface. The large reduction in volume sucks in more steam to the same site and the process continues until the

temperature of the substance raised to that of steam. The condensed water produced moist conditions for killing the microbes present.

The autoclave is a modified pressure cooker which contains a vertical or horizontal cylinder. The cylinder is made up of stainless still. A lid on the cylinder is placed and fastened by screw clams to make it airtight. The lid contains a steam discharge unit, a pressure gauge, and a safety valve. Moreover, a thermostat is present to monitor the temperature. Heat is produced by electricity. At the time of sterilization, the cylinder is filled with an adequate amount of water and it is kept for some time for preheating. After that, the materials which are needed to be sterilized are inserted into the cylinder and the lid is then closed tightly. The temperature will increase eventually along with the pressure. When the temperature reaches 121.1^0C and the pressure at 15 psi the sterilization is performed for 15 minutes.

Uses: Sterilization of

- Culture media, rubber material, dressing gloves.
- Materials that are unable to withstand dry heat in a hot air oven.

Sterilization control

- Thermocouple
- Bacterial spores of Bacillus stearothermophilus used at test organisms.
- Browne's tubes contain red solution which turns into green when exposed to the specific temperature for 15 minutes in an autoclave.
- Autoclave tips.

Ozone

Ozone sterilizer uses oxygen, water, and electricity to produce ozone within the sterilizer and provide sterilization without producing toxic chemicals. It runs at $25\text{-}35^0$C temperature. Inside this device, the oxygen is converted into atomic oxygen due to the intense electrical field. The atomic oxygen is then combined with the oxygen molecule to produce ozone. The ozone provides a sterility assurance of 10^{-6} in approximately 4 hours.

Filtration

This process is useful for sterilizing those materials which are unable to withstand heat. There are several types of filters such as

- *Candle filter*: Used for purification of water. These filters consist of hollow candles and water passes through the candles for purification.
- *Asbestos disc filters*: These are made up of magnesium silicate.
- *Sintered glass filters*: These are prepared by fusing finely powdered glass powders.

- *Membrane filters*: These are made up of cellulose esters and are used for water analysis, sterility testing and for the preparation of the solutions. Membrane filters are available in pore size 0.015 to 12 micron. The .22 micron filter is most commonly used as it is smaller than bacteria.
- *Air filters*: These filters are used in laminar airflow chambers to give bacteria-free air supply. These are also known as High-efficiency particulate air (HEPA) filters. These filters can separate particles of 0. Micron or larger.
- *Syringe filters*: Syringes fitted with the membrane of different diameters are available.

A limitation of using the filtration process is that the pores are not small enough for viruses.

Radiation

Ionizing radiation

Ionizing radiations such as gamma rays, X rays, and cosmic rays are used for sterilization process. Due to the high penetrating power, these radiations are lethal for cells. The bacterial cells are killed by damage in the DNA. Gamma radiations from a cobalt 60 source are commercially used for sterilization of disposable items. This procedure is also known as cold sterilization.

Non-ionizing radiation

Infrared radiation and UV radiation comes under this of radiation. Infrared radiation is used for mass sterilization of syringes and catheters. UV radiation with a wavelength of 240nm to 280nm has bactericidal capacity. The UV radiation causes protein denaturation and interferes with DNA replication of bacteria. UV radiations are used for sterilization of close areas, surfaces, operation theaters, laminar airflow, etc.

Chemical Method

Several chemical agents are used as antiseptic and disinfectants. The properties of a chemical antiseptic or disinfectant are following

- The chemical disinfectants need to have a broad spectrum of activity against all microorganisms such as bacteria, viruses, protozoa and fungi.
- The chemical agents should act in the presence of organic matter.
- High penetration power is an important property of the chemical agents
- The chemical agent needs to be chemically stable under both acidic and basic environments.
- The chemical substances should not have any corrosion activity in metals.
- The disinfectants are needed to be non-toxic if absorbed into circulation.
- Finally, the chemical agents are needed to be easily available and less expensive.

Alcohols

Ethyl alcohol and isopropyl alcohol are frequently used as chemical agents for disinfection. Both of the chemicals facilitate the protein denaturation of bacterial proteins. 70% ethyl alcohol is the standard

concentration which is used for disinfection. These are used as skin antiseptics. Apart from this methyl alcohol has activity against fungal spores and used to disinfection of inoculation cabinets.

Aldehydes

Formaldehyde

It is known for its bactericidal, sporicidal and virucidal activities. It can be used in both aqueous and gaseous form. A 10% formalin solution is a standard chemical disinfectant. It is used for

- Prevention of tissues for histological examinations.
- Sterilization of bacterial vaccines
- Preparation of toxoids from toxins.

Glutaraldehyde

It has its activity against bacteria (*Mycobacterium tuberculosis)*, fungi and viruses (including HIV, hepatitis B, etc). It can also kill spores and is known for its less toxic nature. It is used as a 2% buffered solution. Glutaraldehyde is used for

- Sterilization of cystoscopes, endoscopes, and bronchoscopes
- Sterilization of plastic endotracheal tubes, face masks, metal instruments, etc.

Orthophathalaldehyde

Orthophathalaldehyde (OPA) is a high-level disinfectant and is known for its stability during storage. It has bactericidal effects against mycobacteria. 0.5% OPA is slowly sporicidal and OPA vapors irritate the respiratory tract and eyes, therefore, it must be handled with appropriate safety.

Phenols

Lister (father of antiseptic surgery) used phenol for the first time in the sterilization of surgical instruments. Phenols work as a disinfectant and kill microorganisms by cell membrane damage. It is toxic for the skin. Different derivatives of phenol are used as antiseptics which are following

Cresols

An example of cresol is Lysol which is mostly used for sterilization of infected glasswares, floors, etc.

Chlorhexidine

Savlon is an example of a chlorhexidine solution which is widely used in wounds, preoperative disinfection of the skin. It is bactericidal at high dilution. Moreover, it also has fungicidal activity.

Chloroxylenol

Dettol is commercially available as a chloroxylenol solution. It is less toxic and less irritant.

Hexachlorophene

It is bacteriostatic at very high dilution.

Halogens

Chlorine and iodine are commonly used disinfectants. Chlorine is used in water supplies, swimming pools, food, and dairy industries. Chlorine compounds in the form of bleaching powder, sodium hypochlorite, and chloramines. The disinfection action of all the chlorine compounds is due to the release of free chlorine which becomes a strong oxidative agent.

Iodine in alcoholic and aqueous solution is used as a skin disinfectant. It is active against *M tuberculosis* and slightly active against spores. Compounds with iodine with surface-active agents known as iodophors are claimed to be more active than aqueous or alcohol solution.

Oxidizing agents

Hydrogen peroxide

It is effective against most organisms in the concentration of 3-6 %. However, it kills spores at higher concentrations (10-25%). The mode of action is by the liberation of free hydroxyl radical on the decomposition of hydrogen peroxide. These free radicals are active ingredients in the disinfection process.

Peracetic acid

It is an oxidizing agent and is a more potent germicidal agent than hydrogen peroxide.

Salts

Slats of heavy metals have a toxic effect on bacteria. The salts of copper, silver, and mercury are used as a disinfectant. They are protein coagulant ant act by combining with sulphydryl groups of bacterial proteins and other essential intracellular compounds. Merthiolate (sodium ethyl mercurithiosalicylate) is used in a dilution of 1:10000 for the preservation of sera.

Dyes

Two groups of dyes, aniline and acridine dyes have been used as a skin and wound antiseptics. Both the dyes have bacteriostatic activity. Aniline dyes include crystal violet, brilliant green, and malachite green. Acridine dyes include acriflavine, cuflavin, proflavin, and aminacrine.

Vapor phase Disinfectants

Ethylene Oxide (ETO)

It is a colorless liquid with a boiling point of 10.7^0C. It is effective against all types of microorganisms including viruses and spores. It acts by alkylating the amnio carboxyl, hydroxyl and sulphydryl groups in protein molecules. In addition, it reacts with DNA and RNA. It is specially used for sterilizing plastic and rubber articles, respirators, heart-lung machines, dental equipment, etc.

Betapropilolactone (BPO)

This is a condensation product of ketane and formaldehyde. It has rapid action and used in0.2%. It is more efficient in fumigation than formaldehyde. BPO is used for the inactivation of vaccines

What is Culture Media?

The media is a source of nutrients to support the growth of the micro-organisms in-vitro. The media helps in the growth and counting of microbial cells, selection of microorganisms, and survival of microorganisms. The culture medium can be liquid or gel.

Common ingredients of culture media

- **Peptone-** source of carbon and nitrogen.
- **Beef extract-** source of amino acid, vitamins, minerals.
- **Yeast extract-** source of vitamin, carbon, nitrogen.
- **Distilled water**
- **Agar-** solidifying agent.

How to prepare culture media?

1. Weigh the amount of ingredients powder on weighing machine.
2. Dissolve the ingredients in distilled water.
3. Adjust PH of the medium if needed.
4. Add agar and boiled it to dissolve.
5. Pour the media into flask.
6. Autoclave the media when ingredients fully dissolve.
7. Sterilization is done in autoclave to prevent from contamination, at 121ºC for 15 min at 15lbs.
8. After the autoclave place the media flask in laminar air flow.
9. Sterilize the laminar air flow with 70% alcohol.
10. A bit cools down the media and pours into sterile Petri-plates for solidification.
11. Then sample is ready to spread(spreader) / streak
12. (Inoculation loop) on the medium for identification or isolation of microbes.
13. Sealed the Petri plates with paraffin, label them.
14. Keep them inverted in incubator at 37ºC for 24hrs.
15. Observe the result next day colonies formation is visible on the media.

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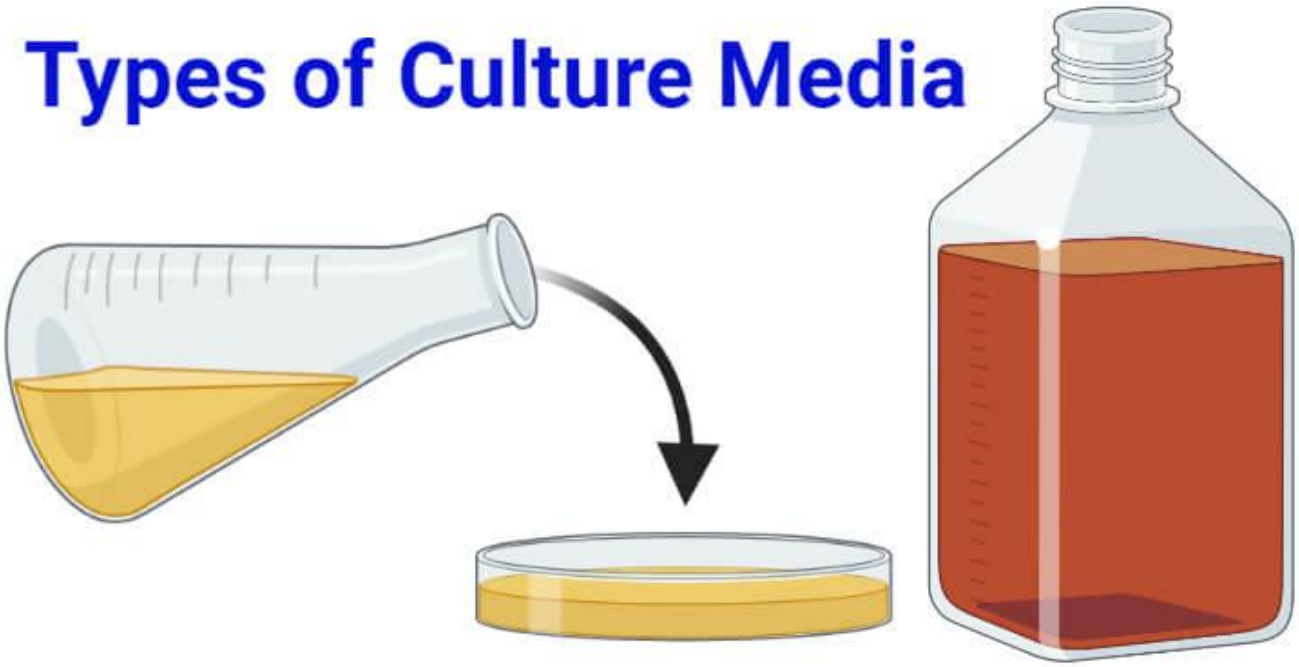


Types of culture media based on consistency/ physical state

1. Solid medium

2. Semi-solid medium

3. Liquid medium

1. Solid media

Principle of Solid Media

It is for the isolation of bacteria as a pure culture on a solid medium.

Robert Koch realized the use of solid media.

Agar is used to hardening the media at 1.5- 2.0% concentration. Solid media allows the growth of bacteria as colonies by streaking on the medium. It solidified at 37 degrees Celsius.

Agar is an un-branched polysaccharide extracted from red algae species like Gelidium. Colonies identification is done on this medium.

Examples of Solid Media

Nutrient agar, MacConkey agar, Blood agar, Chocolate agar.

Growth of bacteria on solid medium appear as smooth, rough, mucoid, round, irregular, filamentous, punctiform.

2. Semi-solid media

Principle of Semi-solid media

This media shows the motility of bacteria and the cultivation of microaerophilic bacteria. This media has agar at a concentration of 0.5% or less. It has a jelly consistency.

Examples of Semi-solid media

Stuart's and Amies media, Hugh and Leifson's oxidation fermentation medium, and Mannitol motility media.

The growth of bacteria in semi-solid appears as a thick line in the medium.

3. Liquid media

Principle of Liquid media

This media shows the growth of a large number of bacteria.

It is called Broth that allows bacteria to grow uniformly with turbidity. The growth occurs at 37ºC in an incubator for 24hrs.

Liquid media don't have the addition of agar; it is for fermentation studies.

Examples of Liquid media

Nutrient broth, Tryptic soy broth, MR-VP broth, phenol red carbohydrate broth.

Growth of bacteria in liquid media- Turbidity is seen at the end of the broth.

Types of culture media based on chemical composition/application

There are seven routine laboratory media.

1. **Basal media**
2. **Enriched media**
3. **Selective media**
4. **Enrichment media**
5. **Indicator media or differential media**
6. **Transport media**
7. **Storage media**

1. Basal media

This media is simple as it enhances the growth of many microorganisms. It's a routinely used medium in the lab, having Carbon and Nitrogen. This media allows the growth; of non- fastidious bacteria without any enrichment source; used for sub-culturing. It's a non-selective medium.

Staphylococcus and *Enterobacteriaceae* grow in this media.

Examples of Basal media

Nutrient Agar, Peptone water.

2. Enriched media

This media requires the addition of other substances like blood, egg, or serum. An enriched media allows the growth of devised microorganisms but inhibits other and fastidious microbes grow as they require nutrients like vitamins and growth-promoting substances.

Example of Enriched media

Blood agar, Chocolate agar, LSS, Monsor's taurocholate, Lowenstein Jensen media. Blood agar identifies hemolytic bacteria, chocolate media for *N. gonorrhea.*

3. Selective media

As by name, we can tell, this media shows the growth of selective; microbes or desired microorganisms and inhibits the growth of unwanted microbes. The inhibition occurs by adding bile salts, antibiotics, dyes, PH adjustments. Media is agar-based; any media is possible to transform into selective by adding inhibitory agar.

UNIT-III

Viral Morphology

Viruses are **acellular**, meaning they are biological entities that do not have a cellular structure. They therefore lack most of the components of cells, such as organelles, ribosomes, and the plasma membrane. A virion consists of a nucleic acid core, an outer protein coating or capsid, and sometimes an outer **envelope** made of protein and phospholipid membranes derived from the host cell. Viruses may also contain additional proteins, such as enzymes. The most obvious difference between members of viral families is their morphology, which is quite diverse. An interesting feature of viral complexity is that the complexity of the host does not correlate with the complexity of the virion. Some of the most complex virion structures are observed in bacteriophages, viruses that infect the simplest living organisms, bacteria.

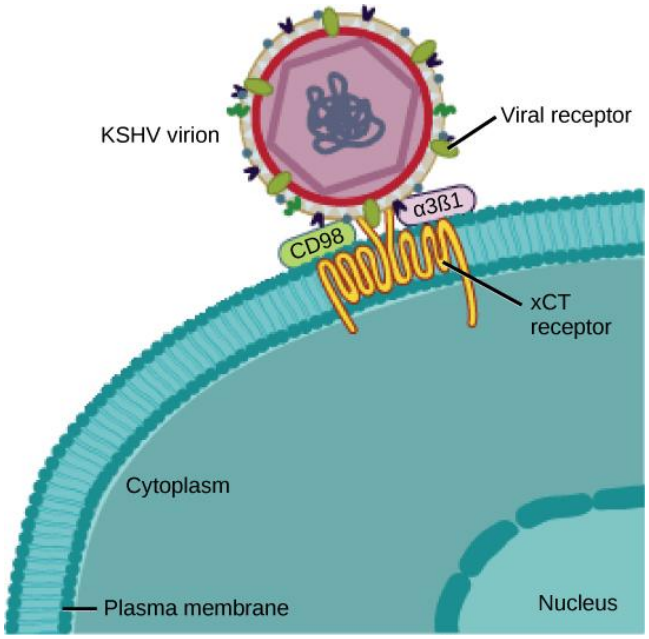
Morphology

Viruses come in many shapes and sizes, but these are consistent and distinct for each viral family. All virions have a nucleic acid genome covered by a protective layer of proteins, called a **capsid**. The capsid is made up of protein subunits called **capsomeres**. Some viral capsids are simple polyhedral "spheres," whereas others are quite complex in structure.

In general, the shapes of viruses are classified into four groups: filamentous, isometric (or icosahedral), enveloped, and head and tail. Filamentous viruses are long and cylindrical. Many plant viruses are filamentous, including TMV. Isometric viruses have shapes that are roughly spherical, such as poliovirus or herpesviruses. Enveloped viruses have membranes surrounding capsids. Animal viruses, such as HIV, are frequently enveloped. Head and tail viruses infect bacteria and have a head that is similar to icosahedral viruses and a tail shape like filamentous viruses.

Many viruses use some sort of glycoprotein to attach to their host cells via molecules on the cell called **viral receptors** ([link]). For these viruses, attachment is a requirement for later penetration of the cell membrane, so they can complete their replication inside the cell. The receptors that viruses use are molecules that are normally found on cell surfaces and have their own physiological functions. Viruses have simply evolved to make use of these molecules for their own replication. For example, HIV uses the CD4 molecule on T lymphocytes as one of its receptors. CD4 is a type of molecule called a cell adhesion molecule, which functions to keep different types of immune cells in close proximity to each other during the generation of a T lymphocyte immune response.

The KSHV virus binds the xCT receptor on the surface of human cells. xCT receptors protect cells against stress. Stressed cells express more xCT receptors than non-stressed cells. The KSHV virion causes cells to become stressed, thereby increasing expression of the receptor to which it binds. (credit: modification of work by NIAID, NIH)



Among the most complex virions known, the T4 bacteriophage, which infects the *Escherichia coli* bacterium, has a tail structure that the virus uses to attach to host cells and a head structure that houses its DNA.

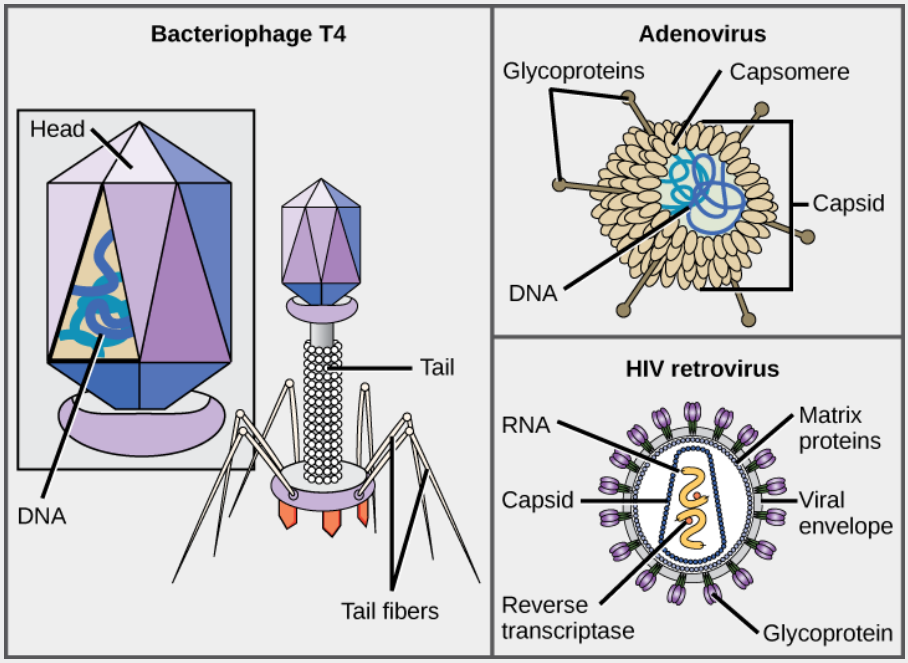
Adenovirus, a non-enveloped animal virus that causes respiratory illnesses in humans, uses glycoprotein spikes protruding from its capsomeres to attach to host cells. Non-enveloped viruses also include those that cause polio (poliovirus), plantar warts (papillomavirus), and hepatitis A (hepatitis A virus).

Enveloped virions like HIV, the causative agent in AIDS, consist of nucleic acid (RNA in the case of HIV) and capsid proteins surrounded by a phospholipid bilayer envelope and its associated proteins. Glycoproteins embedded in the viral envelope are used to attach to host cells. Other envelope proteins are the **matrix proteins** that stabilize the envelope and often play a role in the assembly of progeny virions. Chicken pox, influenza, and mumps are examples of diseases caused by viruses with envelopes. Because of the fragility of the envelope, non-enveloped viruses are more resistant to changes in temperature, pH, and some disinfectants than enveloped viruses.

Overall, the shape of the virion and the presence or absence of an envelope tell us little about what disease the virus may cause or what species it might infect, but they are still useful means to begin viral classification ([link]).

Art Connection

Viruses can be either complex in shape or relatively simple. This figure shows three relatively complex virions: the bacteriophage T4, with its DNA-containing head group and tail fibers that attach to host cells; adenovirus, which uses spikes from its capsid to bind to host cells; and HIV, which uses glycoproteins embedded in its envelope to bind to host cells. Notice that HIV has proteins called matrix proteins, internal to the envelope, which help stabilize virion shape. (credit "bacteriophage, adenovirus": modification of work by NCBI, NIH; credit "HIV retrovirus": modification of work by NIAID, NIH)



Which of the following statements about virus structure is true?

1. All viruses are encased in a viral membrane.
2. The capsomere is made up of small protein subunits called capsids.
3. DNA is the genetic material in all viruses.
4. Glycoproteins help the virus attach to the host cell.

<!–<para>D–>

Types of Nucleic Acid

Unlike nearly all living organisms that use DNA as their genetic material, viruses may use either DNA or RNA as theirs. The **virus core** contains the genome or total genetic content of the virus. Viral genomes tend to be small, containing only those genes that encode proteins that the virus cannot get from the host cell. This genetic material may be single- or double-stranded. It may also be linear or circular. While most viruses contain a single nucleic acid, others have genomes that have several, which are called segments.

In DNA viruses, the viral DNA directs the host cell's replication proteins to synthesize new copies of the viral genome and to transcribe and translate that genome into viral proteins. DNA viruses cause human diseases, such as chickenpox, hepatitis B, and some venereal diseases, like herpes and genital warts.

RNA viruses contain only RNA as their genetic material. To replicate their genomes in the host cell, the RNA viruses encode enzymes that can replicate RNA into DNA, which cannot be done by the host cell. These RNA polymerase enzymes are more likely to make copying errors than DNA polymerases, and therefore often make mistakes during transcription. For this reason, mutations in RNA viruses occur more frequently than in DNA viruses. This causes them to change and adapt more rapidly to their host. Human diseases caused by RNA viruses include hepatitis C, measles, and rabies.

Virus Classification

To understand the features shared among different groups of viruses, a classification scheme is necessary. As most viruses are not thought to have evolved from a common ancestor, however, the methods that scientists use to classify living things are not very useful. Biologists have used several classification systems in the past, based on the morphology and genetics of the different viruses. However, these earlier classification methods grouped viruses differently, based on which features of the virus they were using to classify them. The most commonly used classification method today is called the Baltimore classification scheme and is based on how messenger RNA (mRNA) is generated in each particular type of virus.

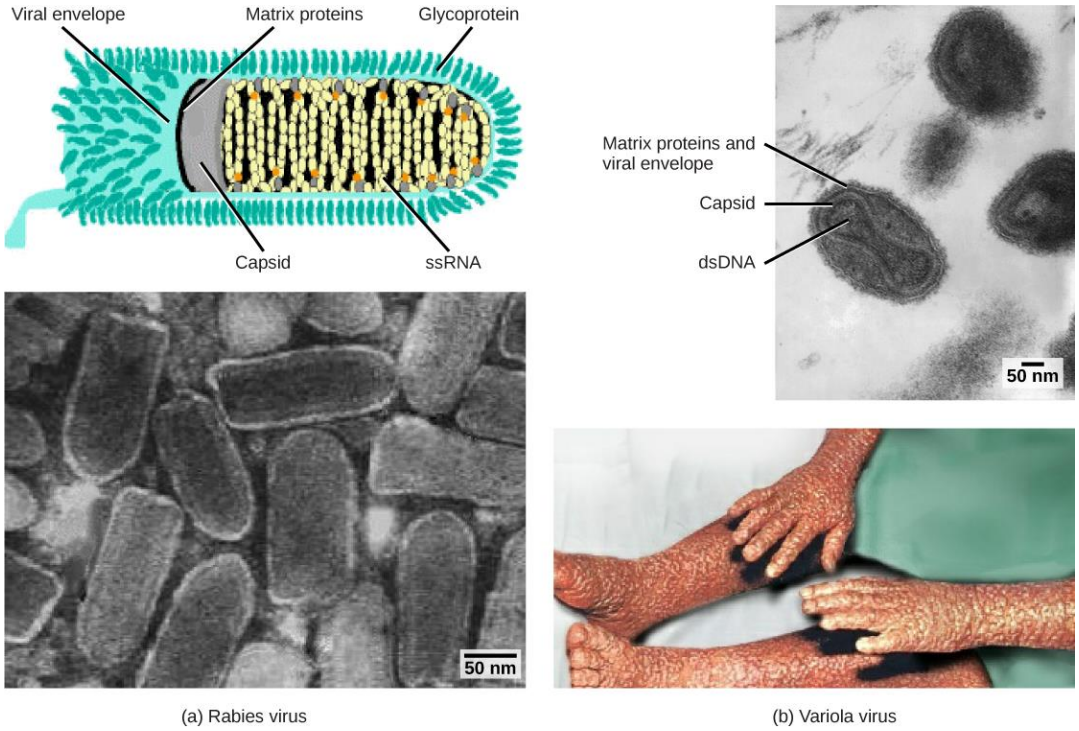
Past Systems of Classification

Viruses are classified in several ways: by factors such as their core content ([link] and [link]), the structure of their capsids, and whether they have an outer envelope. The type of genetic material (DNA or RNA) and its structure (single- or double-stranded, linear or circular, and segmented or non-segmented) are used to classify the virus core structures.

Virus Classification by Genome Structure and Core

Core Classifications	Examples
RNA	Rabies virus, retroviruses
DNA	Herpesviruses, smallpox virus
Single-stranded	Rabies virus, retroviruses
Double-stranded	Herpesviruses, smallpox virus
Linear	Rabies virus, retroviruses, herpesviruses, smallpox virus
Circular	Papillomaviruses, many bacteriophages
Non-segmented: genome consists of a single segment of genetic material	Parainfluenza viruses
Segmented: genome is divided into multiple segments	Influenza viruses

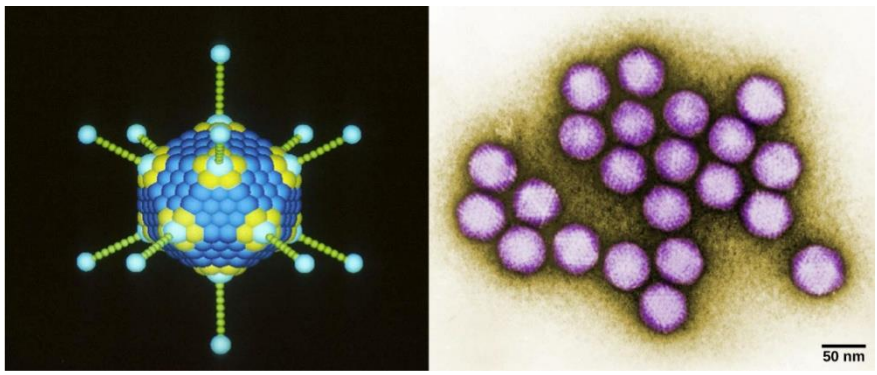
Viruses are classified based on their core genetic material and capsid design. (a) Rabies virus has a single-stranded RNA (ssRNA) core and an enveloped helical capsid, whereas (b) variola virus, the causative agent of smallpox, has a double-stranded DNA (dsDNA) core and a complex capsid. Rabies transmission occurs when saliva from an infected mammal enters a wound. The virus travels through neurons in the peripheral nervous system to the central nervous system where it impairs brain function, and then travels to other tissues. The virus can infect any mammal, and most die within weeks of infection. Smallpox is a human virus transmitted by inhalation of the variola virus, localized in the skin, mouth, and throat, which causes a characteristic rash. Before its eradication in 1979, infection resulted in a 30–35 percent mortality rate. (credit "rabies diagram": modification of work by CDC; "rabies micrograph": modification of work by Dr. Fred Murphy, CDC; credit "small pox micrograph": modification of work by Dr. Fred Murphy, Sylvia Whitfield, CDC; credit "smallpox photo": modification of work by CDC; scale-bar data from Matt Russell)



(a) Rabies virus (b) Variola virus

Viruses can also be classified by the design of their capsids ([link] and [link]). Capsids are classified as naked icosahedral, enveloped icosahedral, enveloped helical, naked helical, and complex ([link] and [link]). The type of genetic material (DNA or RNA) and its structure (single- or double-stranded, linear or circular, and segmented or non-segmented) are used to classify the virus core structures ([link]).

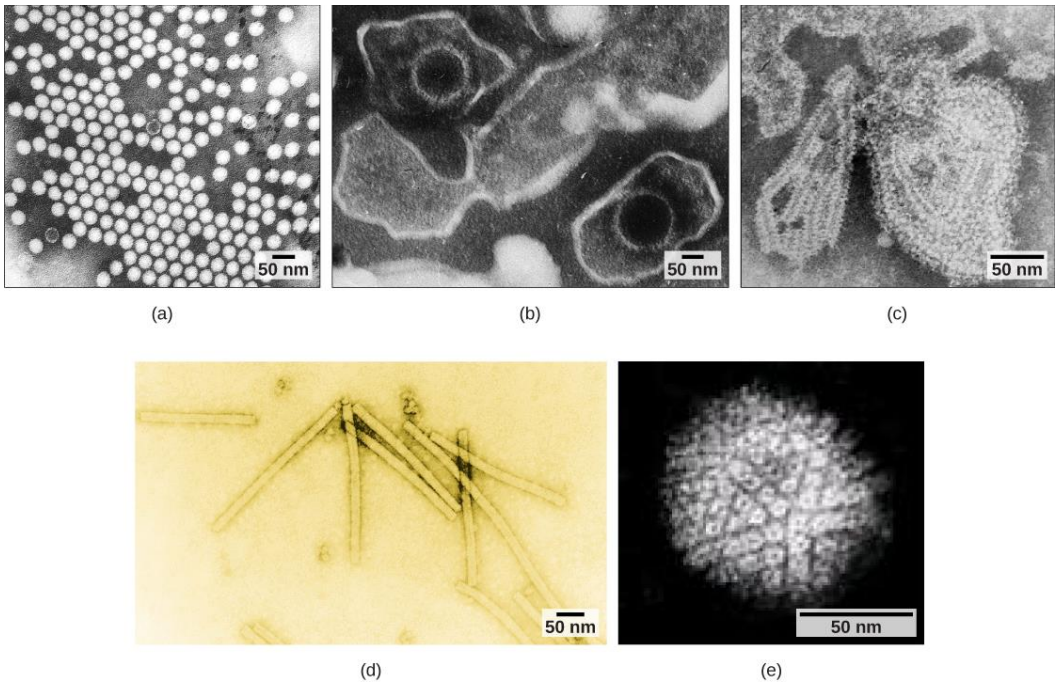
Adenovirus (left) is depicted with a double-stranded DNA genome enclosed in an icosahedral capsid that is 90–100 nm across. The virus, shown clustered in the micrograph (right), is transmitted orally and causes a variety of illnesses in vertebrates, including human eye and respiratory infections. (credit “adenovirus”: modification of work by Dr. Richard Feldmann, National Cancer Institute; credit “micrograph”: modification of work by Dr. G. William Gary, Jr., CDC; scale-bar data from Matt Russell)



Virus Classification by Capsid Structure

Capsid Classification	Examples
Naked icosahedral	Hepatitis A virus, polioviruses
Enveloped icosahedral	Epstein-Barr virus, herpes simplex virus, rubella virus, yellow fever virus, HIV-1
Enveloped helical	Influenza viruses, mumps virus, measles virus, rabies virus
Naked helical	Tobacco mosaic virus
Complex with many proteins; some have combinations of icosahedral and helical capsid structures	Herpesviruses, smallpox virus, hepatitis B virus, T4 bacteriophage

Transmission electron micrographs of various viruses show their structures. The capsid of the (a) polio virus is naked icosahedral; (b) the Epstein-Barr virus capsid is enveloped icosahedral; (c) the mumps virus capsid is an enveloped helix; (d) the tobacco mosaic virus capsid is naked helical; and (e) the herpesvirus capsid is complex. (credit a: modification of work by Dr. Fred Murphy, Sylvia Whitfield; credit b: modification of work by Liza Gross; credit c: modification of work by Dr. F. A. Murphy, CDC; credit d: modification of work by USDA ARS; credit e: modification of work by Linda Stannard, Department of Medical Microbiology, University of Cape Town, South Africa, NASA; scale-bar data from Matt Russell)



Baltimore Classification

The most commonly used system of virus classification was developed by Nobel Prize-winning biologist David Baltimore in the early 1970s. In addition to the differences in morphology and genetics mentioned above, the Baltimore classification scheme groups viruses according to how the mRNA is produced during the replicative cycle of the virus.

Group I viruses contain double-stranded DNA (dsDNA) as their genome. Their mRNA is produced by transcription in much the same way as with cellular DNA. **Group II** viruses have single-stranded DNA (ssDNA) as their genome. They convert their single-stranded genomes into a dsDNA intermediate before transcription to mRNA can occur. **Group III** viruses use dsRNA as their genome. The strands separate, and one of them is used as a template for the generation of mRNA using the RNA-dependent RNA polymerase encoded by the virus. **Group IV** viruses have ssRNA as their genome with a positive polarity. **Positive polarity** means that the genomic RNA can serve directly as mRNA. Intermediates of dsRNA, called **replicative intermediates**, are made in the process of copying the genomic RNA. Multiple, full-length RNA strands of negative polarity (complimentary to the positive-stranded genomic RNA) are formed from these intermediates, which may then serve as templates for the production of RNA with positive polarity, including both full-length genomic RNA and shorter viral mRNAs. **Group V** viruses contain ssRNA genomes with a **negative polarity**, meaning that their sequence is complementary to the mRNA. As with Group IV viruses, dsRNA intermediates are used to make copies of the genome and produce mRNA. In this case, the negative-stranded genome can be converted directly to mRNA. Additionally, full-length

positive RNA strands are made to serve as templates for the production of the negative-stranded genome. **Group VI** viruses have diploid (two copies) ssRNA genomes that must be converted, using the enzyme **reverse transcriptase**, to dsDNA; the dsDNA is then transported to the nucleus of the host cell and inserted into the host genome. Then, mRNA can be produced by transcription of the viral DNA that was integrated into the host genome. **Group VII** viruses have partial dsDNA genomes and make ssRNA intermediates that act as mRNA, but are also converted back into dsDNA genomes by reverse transcriptase, necessary for genome replication. The characteristics of each group in the Baltimore classification are summarized in [link] with examples of each group.

Baltimore Classification

Group	Characteristics	Mode of mRNA Production	Example
I	Double-stranded DNA	mRNA is transcribed directly from the DNA template	Herpes simplex (herpesvirus)
II	Single-stranded DNA	DNA is converted to double-stranded form before RNA is transcribed	Canine parvovirus (parvovirus)
III	Double-stranded RNA	mRNA is transcribed from the RNA genome	Childhood gastroenteritis (rotavirus)
IV	Single stranded RNA (+)	Genome functions as mRNA	Common cold (pircornavirus)

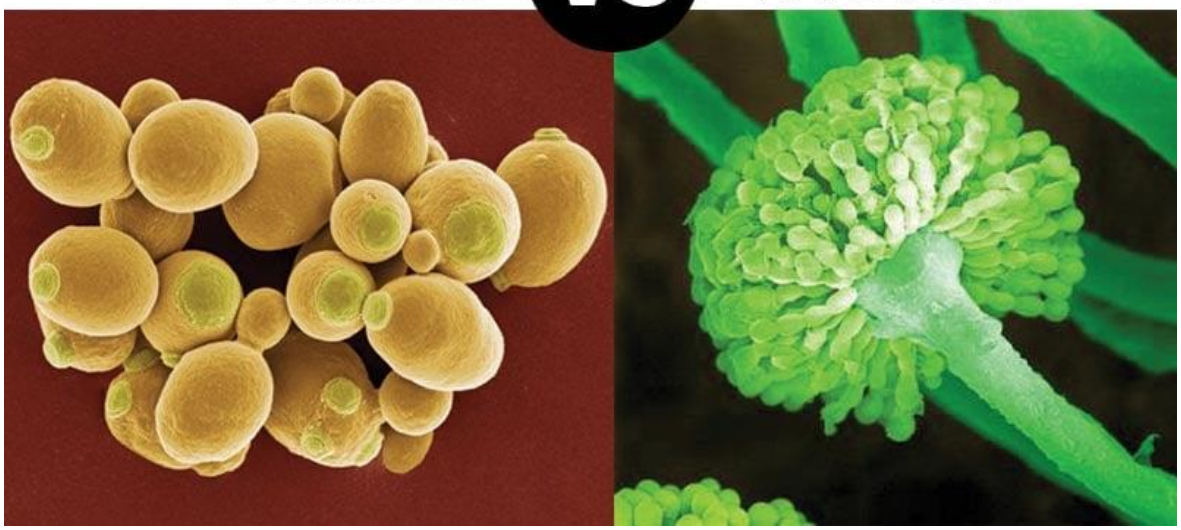
Baltimore Classification

Group	Characteristics	Mode of mRNA Production	Example
V	Single stranded RNA (-)	mRNA is transcribed from the RNA genome	Rabies (rhabdovirus)
VI	Single stranded RNA viruses with reverse transcriptase	Reverse transcriptase makes DNA from the RNA genome; DNA is then incorporated in the host genome; mRNA is transcribed from the incorporated DNA	Human immunodeficiency virus (HIV)
VII	Double stranded DNA viruses with reverse transcriptase	The viral genome is double-stranded DNA, but viral DNA is replicated through an RNA intermediate; the RNA may serve directly as mRNA or as a template to make mRNA	Hepatitis B virus (hepadnavirus)

Differences between Yeasts and Molds

Both yeast and molds are eukaryotes – organisms with cell nuclei and membrane-bound organelles in the kingdom Fungi.

Yeasts vs Molds



The major differences between Yeasts and Molds are:

S.N.	Character	Yeasts	Molds
1.	Definition	Yeast is a unicellular, budding fungus.	Mold is a multicellular, threadlike fungus.
2.	Form	Grow as large single cells.	Grow as multiple tubular branches.
3.	Cell type	Unicellular	Multicellular
4.	Habitat	Very common. It can be found on fruit and berries, in the stomachs of mammals and on the skin, among other places.	Typically found in damp, dark or humid areas.
5.	Appearance	White and thready. Usually oval in shape.	Mold has a fuzzy appearance and can be found in several shapes.

6.	Hyphae	Yeasts do not have true hyphae. Instead, they form multicellular structures called pseudo-hyphae.	Molds have microscopic filaments called hyphae.
7.	Spore	Yeast is a not a sporing species of fungi.	Mold is a sporing fungus
8.	Colony morphology	Yeast colonies are soft, opaque and cream-colored.	Filamentous type colony with vegetative hyphae and aerial hyphae.
9.	Color	Yeasts are less colorful compared to molds (colorless).	Molds are very colorful and maybe orange, green, black, brown, pink or purple.
10.	Incubation Temperature	Routine incubation temperature is usually 25ºC to 30ºC (room temperature).	Routine incubation temperature is usually 25º to 30º C, although 35º C incubation can be used to differentiate some molds based on temperature tolerance or to determine whether organisms are diphasic.
11.	Cultivation time	These organisms usually grow within 24 to 36 hours after inoculation on media.	These organisms usually grow more slowly than yeasts after inoculation to media.
12.	Aerobic/Anaerobic	Yeast can grow in aerobic as well as in anaerobic conditions.	Molds grow only in aerobic conditions.

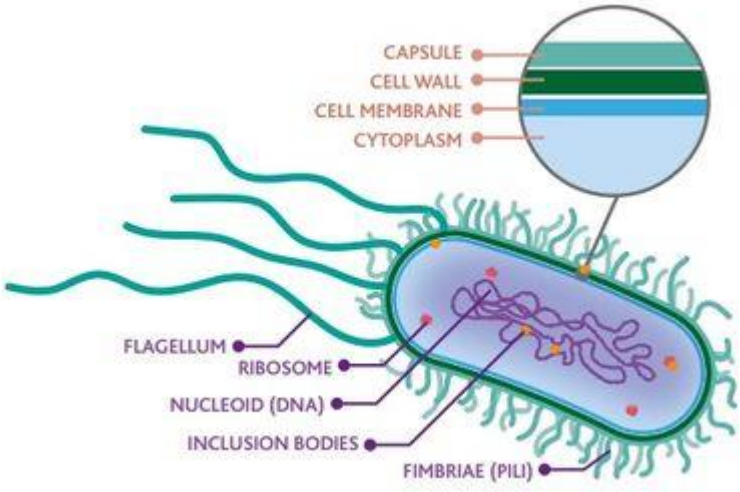
13.	pH range for growth	Growth limited to a pH range of 4.0 to 4.5.	Mold can grow in a wider range of acidity (pH) levels than yeasts.
14.	Diagnosis/ Identification	Identification bases on physiologic tests and a few key morphologic differences.	Most clinical molds can be determined by microscopic examination of the ontogeny and morphology of their asexual spores.
15.	Reproduction	Most reproduce asexually through mitosis. The most common form called “budding.” A smaller number of yeasts reproduce by binary fission.	Reproduce through small spores, which can be either sexual or asexual.
16.	Asexual Spores	Blastospore	Sporangiospores and Conidia
17.	Sexual Spores	No Sexual Spores.	Zygospores, Ascospores, and Basidiospores
18.	Energy Production	Convert carbohydrates to alcohol and carbon dioxide in anaerobic through fermentation. Also, obtain carbon from hexose sugars.	Secrete hydrolytic enzymes that degrade biopolymers such as starch, cellulose, and lignin into simpler substances that can be absorbed.
19.	Health risks	It can cause infection in individuals with compromised immune systems.	It can cause allergic reactions and respiratory problems.

20.	Other risks	Comparatively less involved in spoilage.	Molds cause a greater threat in terms of food spoilage and sanitation concerns, particularly in fresh produce.
21.	Uses	Ethanol production, baking, vitamin supplements, the study of the cell cycle.	Some molds are used in food production, for example, *Penicillium* is used in the production of cheese, *Neurospora* in the production of oncom, which is made from the by-product of tofu. Mold is also a crucial saprophyte.
22.	Species	1500 known species – 1% of all fungi.	There are 400,000 types of molds.
23.	Examples	*Saccharomyces cerevisiae, Cryptococcus neoformans*, etc.	*Alternaria, Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus, Trichophyton*, etc.

UNIT-IV

Bacterial Infections

Introduction



Bacteria

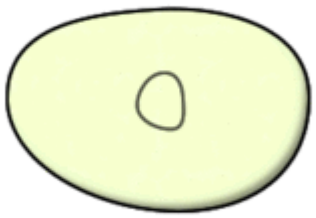
Bacteria are microscopic, single-celled organisms. They are among the earliest known life forms on earth. There are thousands of different kinds of bacteria, and they live in every conceivable environment all over the world[1]. Some are airborne and others are most prevalent in water, soil, plants, animals, and even people.

Many strains of bacteria are harmless and some are even beneficial, such as those found in the human gastrointestinal tract to aid digestion and produce vitamins. There are few (less than 1% of all bacteria types) that cause illness in humans. Some bacteria can be quite dangerous, resulting in salmonella, pneumonia, or meningitis[2].

- The global problem of infectious and deadly diseases caused by bacteria are presently major scientific and medical issues.
- Bacterial infections have a large impact on public health.
- As a general rule, bacterial infections are easier to treat than viral infections, since we have an extensive army of antimicrobial agents with activity against bacteria.
- Bacterial resistance to antimicrobials is a rapidly growing problem with potentially devastating consequences.[3]
- The most deadly bacterial disease contracted by human beings is mycobacterium tuberculosis, the world's leading infectious disease with more than 1,700,000 deaths per year. As much as

13% of cases are resistant to most antibiotics, and about 6% are resistant or unresponsive to essentially all treatment[2].

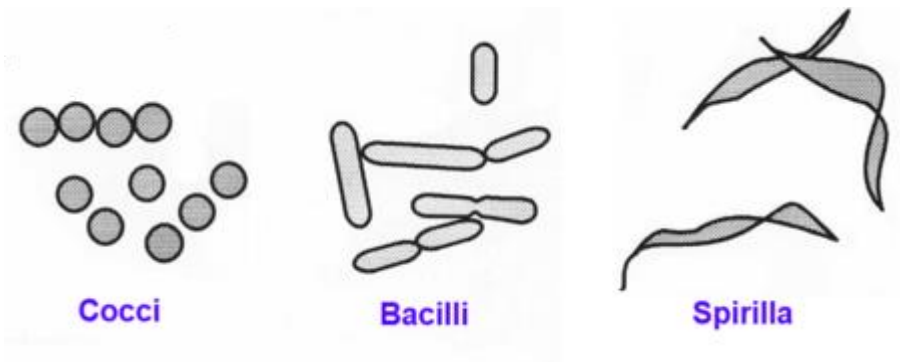
Structure



Binary Fission

Bacteria are prokaryotic organisms that carry their genetic information in a double-stranded circular molecule of DNA. Some species also contain small circular plasmids of additional DNA. The cell cytoplasm contains ribosomes and there is both a cell membrane and, in all species except *Mycoplasma*, a complex cell wall. External to the cell wall, some bacteria have capsules, flagella, or pili. Bacteria normally reproduce by binary fission. Under the proper conditions, some bacteria can divide and multiply rapidly. Consequently, some infections require only a small number of organisms to cause potentially overwhelming infection.

Classification



Bacteria Shapes

Several ways include:

- Staining: Gram-positive or Gram-negative based on the characteristics of their cell wall, as seen under a microscope after stains have been administered, a procedure called Gram staining, that was developed in 1882 by Hans Christian Gram. Most bacteria fall into one of these two categories. One of the main differences between gram-positive and gram-negative organisms is that gram-negative bacteria tend to produce an endotoxin that can cause tissue destruction, shock, and death. The two classes of bacteria differ in their antibiotic susceptibilities as well, and different types of antibiotics are effective against them.
- Need for oxygen: Aerobic or Anaerobic, based on their growth responses in the presence and absence of oxygen[3].
- Scientific names: Bacteria, like other living things, are classified by genus (based on having one or several similar characteristics) and, within the genus, by species. Their scientific name is genus followed by species (for example, Clostridium botulinum). Within a species, there may be different types, called strains. Strains differ in genetic makeup and chemical components. Sometimes certain drugs and vaccines are effective only against certain strains.
- Shapes: All bacteria may be classified as one of three basic shapes: spheres (cocci), rods (bacilli), and spirals or helixes (spirochetes)[1].

Mode of Action

Each species of bacteria has a predilection to infect certain organs and not others. eg. Neisseria meningitidis normally infects the meninges of the central nervous system, it is not, however, a cause of skin infection.

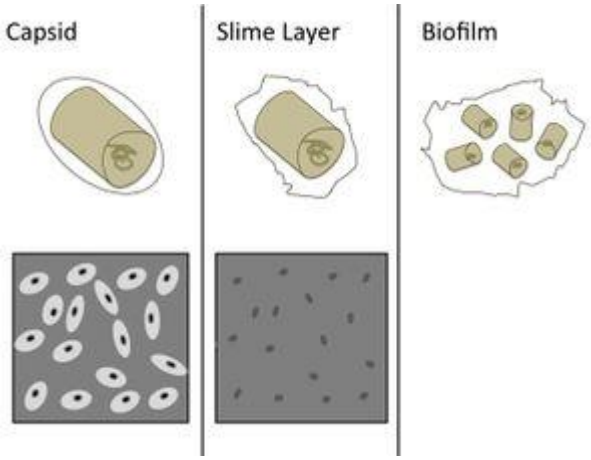
Disease can be caused by the

- Destruction of the body's cells by the organism
- Body's immune response to the infection. Antibiotics may be of little or no use when the disease manifestations are a result of the body's attempts to rid itself of the bacteria. The systemic inflammatory response syndrome (SIRS), usually caused by a bacterial infection, is an overwhelming inflammatory response to infection, manifested by the release of large numbers of cytokines and presenting with signs of infection and early signs of hemodynamic instability.

If allowed to progress, SIRS patients can go on to develop sepsis, with multiorgan failure and death. Once the cascade of events has begun, even the strongest antibiotics are often powerless to stop this progression[3].

The immune response is the way in which your body recognizes and defends itself against bacteria, viruses, and other substances that are foreign and harmful. It is the job of the immune system to protect our bodies from harmful invaders by recognizing and responding to antigens. See the Immune System

Bacterial Defences



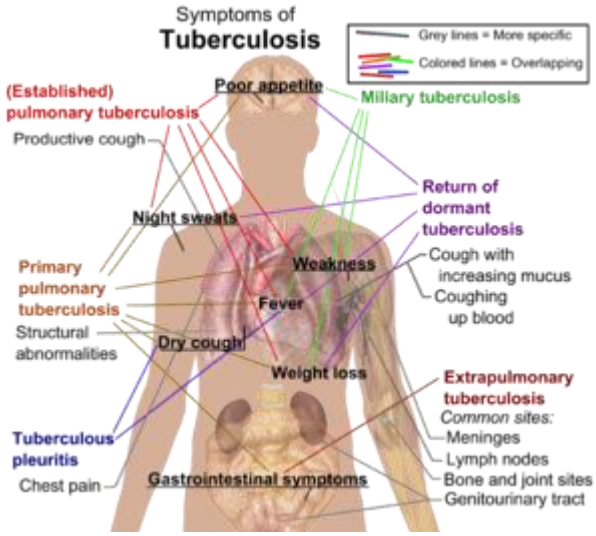
Bacteria Capsules and Slime Layers

Bacteria have many ways of defending themselves.

- Biolfilm
- Capsule: Some bacteria are enclosed in a protective capsule which helps prevent white blood cells from ingesting the bacteria (such bacteria are described as encapsulated).
- Outer membrane: Under the capsule, gram-negative bacteria have an outer membrane that protects them against certain antibiotics. When disrupted, this membrane releases endotoxins. Endotoxins contribute to the severity of symptoms during infections with gram-negative bacteria.
- Spores: an inactive (dormant) form of bacteria. Spores can enable bacteria to survive when environmental conditions are difficult. When conditions are favorable, each spore germinates into an active bacterium.
- Flagella: Long, thin filaments that protrude from the cell surface and enable bacteria to move. Bacteria without flagella cannot move on their own.

- Antibiotic Resistance: Some bacteria are naturally resistant to certain antibiotics. Other bacteria develop resistance to drugs because they acquire genes from other bacteria that have become resistant or because their genes mutate. The genes that encode for drug resistance can be passed to following generations of bacteria or sometimes even to other species of bacteria.[1]

Most Deadly Bacterial Infections

- 

  Tuberculosis

- Anthrax
- Tetanus
- Leptospirosis
- Pneumonia
- Cholera
- Botulism
- Pseudomonas Infection
- MRSA Infection
- E.Coli Infection
- Meningitis
- Gonorrhea
- Bubonic Plague
- Syphilis

Prevention

Measures to prevent infection have a dramatic impact on morbidity and mortality. Prevention is especially important in this age of increasing antibiotic resistance, because treatment can be so difficult to achieve.

See Infection Prevention and Control

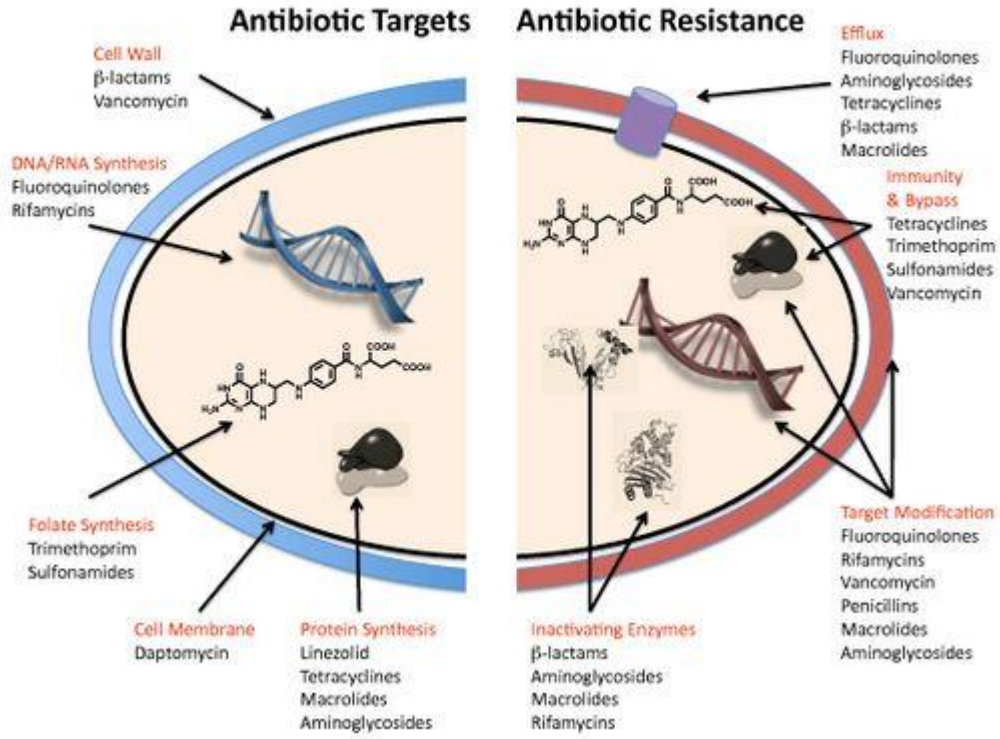
- There are three major principals of control of bacterial infection: Eliminate or contain the source of infection, interrupt the chain of transmission, and protect the host against infection or disease (which measure is most effective often depends on the reservoir for the infection).
- There is increasing recognition that elimination of important cofactors, such as air pollution from vehicles or from indoor cooking, can markedly reduce the incidence of bacterial infections.
- Prevention of infection e.g through a vaccine, is generally called primary prevention, treatment of infected people to prevent symptomatic infection is called secondary prevention, and treatment of infected people to prevent transmission to other humans is called tertiary prevention[3].

An example of poor supervision of a preventive measure is illustrated below.

- Several thousand people in northwest China tested positive for a bacterial disease in an outbreak caused by a leak at a biopharmaceutical company in 2019. This outbreak stemmed from a leak at a biological pharmaceutical factory, which occurred between late July to late August 2019. While producing Brucella vaccines for animal use, the factory used expired disinfectants and sanitizers (meaning not all bacteria were eradicated in the waste gas).
- This contaminated waste gas formed aerosols that contained the bacteria and leaked into the air, carried by wind down to the Veterinary Research Institute, where the outbreak first hit.
- People at the institute began reporting infections in November, and it quickly accelerated. By the end of December, at least 181 people at the institute had been infected with brucellosis[5]

Antibiotic resistance

Diagram R: Showing the ways in which antibiotics can disrupt bacterial processes, and the methods of antibiotic resistance bacteria often use.



- Antibiotic resistance mechanisms

  Antibiotic resistance is one of the biggest threats to global health, food security, and development today.

- Antibiotic resistance can affect anyone, of any age, in any country.
- Antibiotic resistance occurs naturally, but misuse of antibiotics in humans and animals is accelerating the process.
- A growing number of infections – such as pneumonia, tuberculosis, gonorrhoea, and salmonellosis – are becoming harder to treat as the antibiotics used to treat them become less effective.
- Antibiotic resistance leads to longer hospital stays, higher medical costs and increased mortality

Viral Infections

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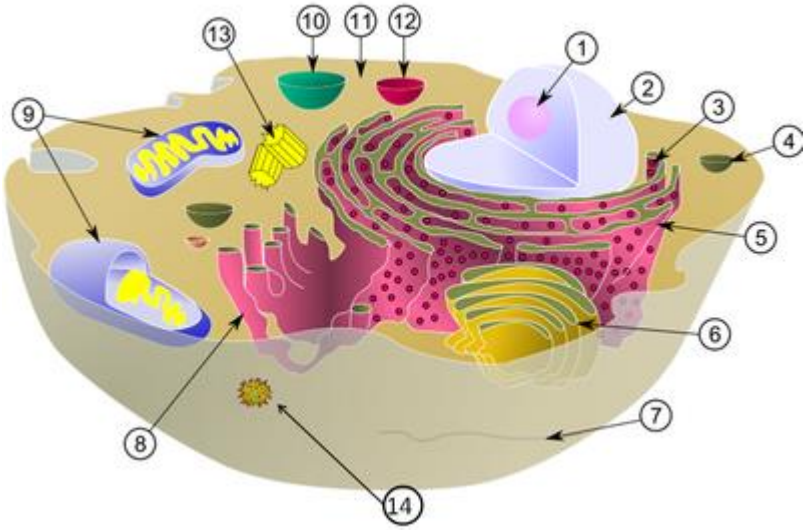


Viral infections are among the most common afflictions of man. It has been estimated that children experience two to seven respiratory infections each year; adults are afflicted with one to three such episodes[1]. Image shows cell with relative size of virus (14)

- Viruses cause familiar infectious diseases such as the common cold, flu and warts. They also cause severe illnesses such as HIV/AIDS, Ebola, influenza and COVID-19.

Viral infections occur due to infection with a virus.

- Millions of different viruses may exist, but researchers have only identified about 5,000 types to date.
- Viruses contain a small piece of genetic code, and a coat of protein and lipid (fat) molecules protects them.

Viruses invade a host and attach themselves to a cell. As they enter the cell, they release their genetic material. This material forces the cell to replicate the virus, and the virus multiplies. The cell may then:

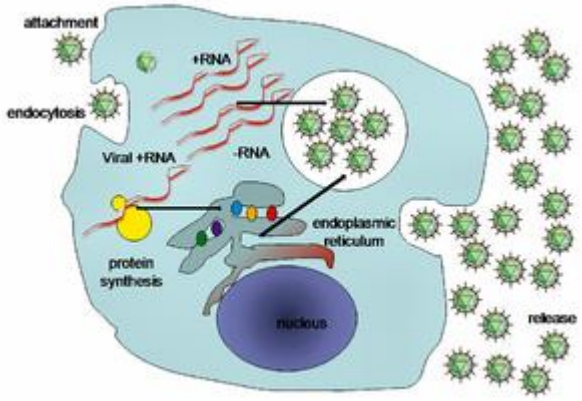
- Die and it releases replicates of the virus, which infect new cells.

- Change the function of the host cell. eg. Some viruses like the human papillomavirus (HPV) and Epstein-Barr virus (EBV), can lead to cancer by forcing cells to replicate in an uncontrolled way.

For most viral infections, treatments can only help with symptoms while you wait for your immune system to fight off the virus. There are antiviral medicines to treat some viral infections. Currently dramatic progress in antiviral therapeutics is occurring[2]. Vaccines can help in prevention of many viral diseases.[3]

- Viruses may remain dormant for a period before multiplying again. The person with the virus can appear to have fully recovered, but they may get sick again when the virus reactivates[4].

Viral Infections



A few notable examples that have garnered the attention of the public health community and the population at large include: COVID 19, Ebola, SARS, Influenza, Zika, Yellow fever, Human immunodeficiency virus (HIV / AIDS), Human papillomavirus (HPV), Viral gastroenteritis, Varicella, and Viral hepatitis[5].

Respiratory Infections

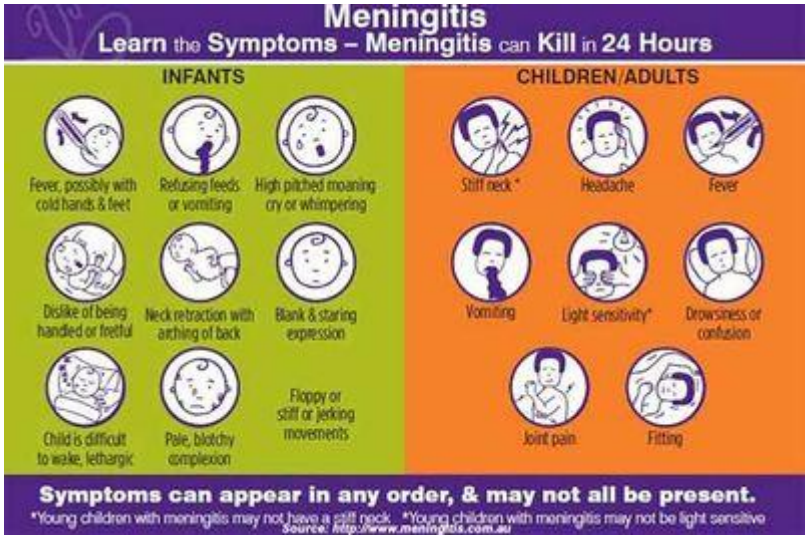
A variety of viruses cause different types of respiratory infections.

- Rhinovirus, coronavirus and adenovirus are the leading causes of the common cold.
-

Influenza viruses infect the upper respiratory system and sometimes spreads to the lungs causing pneumonia. Influenza has been and continues to be one of the great scourges of man. Influenza viruses produce epidemic disease annually. Irregularly, but with all-too-great frequency, widespread epidemics of influenza occur, occasionally producing a pandemic that involves virtually the whole world. Epidemics attributed to the influenza viruses have occurred throughout recorded history. In the past century, major epidemics occurred in 1890, 1900, 1918, 19S7, 1968 and 2019. The great pandemic of influenza in 1918-1919 is estimated to have killed 20--40 million people and accounted for 80% of the deaths in the U.S. Army during World War I.[1]

- Another virus called the respiratory syncytial virus (RSV) causes a respiratory infection called bronchiolitis in infants and toddlers[6].

Central Nervous System Infections



Several viruses can infect the central nervous system (brain and spinal cord).

Viral CNS infections have an annual incidence ranging from 0.26 to 17 cases per 100,000 depending on the age and vaccination status of the population.

Enteroviruses (a genus of positive-sense single-stranded RNA viruses, so named by their transmission-route through the intestine) are the most common cause of viral CNS infection (nearly 60%), followed by arbovirus and herpes virus, such as herpes simplex virus (HSV) and varicella zoster virus (VZV)[7].

Viral Meningitis:

- Enteroviruses are responsible for 80 to 90% and mumps for 10 to 20% of diagnosed cases of viral meningitis, with many other viruses sometimes incriminated with considerable geographical and seasonal variation.

Viral Encephalitis

- Japanese encephalitis is the commonest cause of encephalitis in Asia: other causes—with considerable geographical and seasonal variation—include dengue viruses, Enteroviruses (EV71) rabies, Nipah virus, herpes simplex, West Nile virus, and mumps.

Viral Myelitis

- Viral 'paralytic' myelitis is classically caused by poliovirus, which has now been virtually eliminated from the Americas: other causes—with considerable geographical and seasonal variation—include Japanese encephalitis and various coxsackieviruses, echoviruses, enteroviruses and flaviruses.[8]

Skin Infections

Viruses cause a wide array of skin infections. eg.

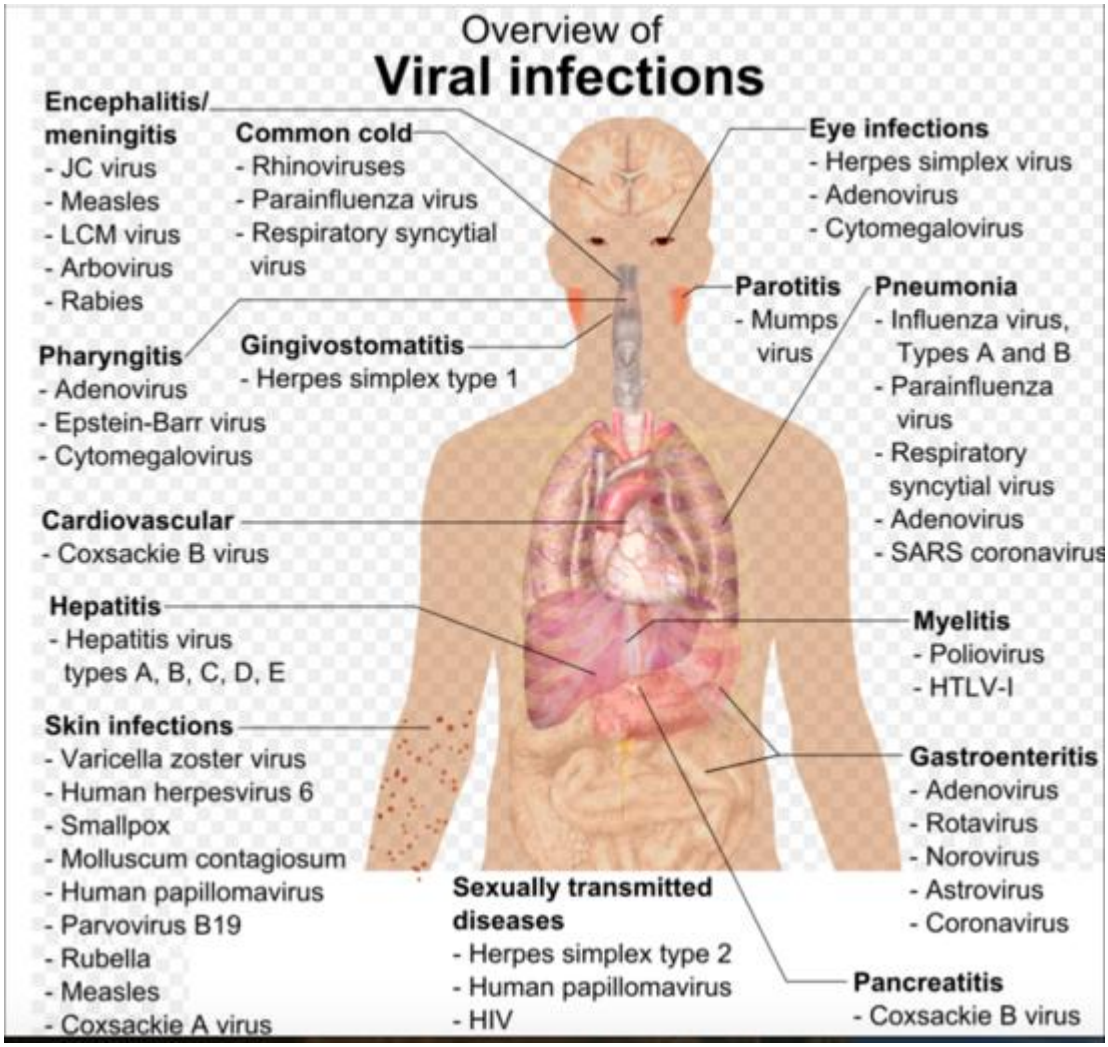
- Herpes simplex viruses (HSV) cause some of the most common skin infections. HSV type 1 tends to cause vesicles in the mouth and on the lips, commonly known as cold sores or fever blisters. HSV type-2 tends to cause genital herpes.
- The varicella virus causes chickenpox, an illness characterized by itchy fluid-filled bumps on the skin that eventually rupture and scab over. The varicella virus also causes shingles, which is a reactivation of the virus years after the initial bout of chickenpox.
- Human papillomaviruses (HPV), cause warts[6].

Digestive System Infections

Several types of viruses cause viral gastroenteritis, commonly called the stomach flu.

- This common illness, characterized by diarrhea, nausea and vomiting, is caused by many different viruses, but not the influenza virus
- According to a June 2012 "American Family Physician" article, viruses cause 75 to 90 percent of acute gastrointestinal disease in children[9]

Diagnosis



Viral infections are causing serious problems in human population worldwide - take the recent outbreak of coronavirus disease 2019. The first step in combating viral pathogens is to get a timely and accurate diagnosis. Early and accurate detection of the viral presence is crucial for appropriate treatment, control, and prevention of epidemics[10].

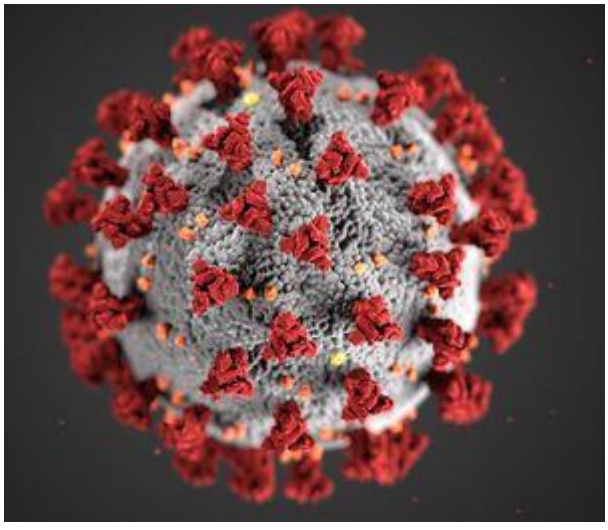
Diagnostic virology has now entered the mainstream of medical practice as a result of several independent developments.

- The dramatic progress in antiviral therapeutics has increased the need for specific viral diagnoses.
- Technological developments, particularly in the area of nucleic acid chemistry, have provided important new tools for viral diagnosis.
- The number of patients at risk for opportunistic viral infections has expanded greatly as a result of the HIV/AIDS epidemic.
- Modern management of HIV infection and hepatitis C is providing a new paradigm for the integration of molecular techniques into management of chronic viral infections.

These developments are not only increasing the use of diagnostic virology but are reshaping the field.

Multiple methods are used for the laboratory diagnosis of viral infections, including viral culture, antigen detection, nucleic acid detection, and serology. The role of culture is diminishing as new immunologic and molecular tests are developed that provide more rapid results and are able to detect a larger number of viruses[2]

Emergence of Viral Diseases



In order for a new viral disease to emerge, the causative virus must infect and successfully invade its host, bypassing the complex and sophisticated antiviral defenses that have evolved in all animals. It is to be stressed that necessary host, virological, and environmental factors must, typically, coincide for a disease to emerge[11].

Many of the scariest viruses that have caused past or current epidemics originated in other animals and then jumped to people: HIV from other primates, influenza from birds and pigs, and Ebola probably from bats. So, too, for coronaviruses: The ones behind SARS (severe acute respiratory syndrome), MERS (Middle East respiratory syndrome) and Covid-19 all probably originated in bats and arrived in people via another, stepping-stone species, likely palm civets, camels and possibly pangolins, respectively.

Making the jump from one species to another isn't easy

- Successful viruses have to be tightly adapted to their hosts.
- To get into a host cell, a molecule on the virus's surface has to match a receptor on the outside of the cell, like a key fitting into a lock.
- Once inside the cell, the virus has to evade the cell's immune defenses and then commandeer the appropriate parts of the host's biochemistry to churn out new viruses.
- Any or all of these factors are likely to differ from one host species to another, so viruses will need to change genetically — that is, evolve — in order to set up shop in a new animal.

Host switching actually involves two steps

- The virus has to be able to invade the new host's cells: That's a minimum requirement for making the host sick.
- To become capable of causing epidemics, the virus also has to become infectious — that is, transmissible between individuals — in its new host. That's what elevates a virus from an occasional nuisance to one capable of causing widespread harm