

**Sporozoa** undergo a complex life cycle with alternating sexual and asexual reproductive phases. The human parasites *Cryptosporidium*, *Cyclospora*, and *Toxoplasma* and the malarial parasites (*Plasmodium* species) are all intracellular parasites.

### ***Plasmodium* Species (Blood Sporozoa)**

Phylum: Apicomplexa

Class: sporozoa

Malaria is the number one killer of all the parasitic diseases. It is estimated that at least 1 million people die of malaria each year, mostly children under 5 years of age. Transmission to humans is by the bloodsucking bite of female *Anopheles* mosquitoes. Four species of *Plasmodium* cause malaria in humans: ***Plasmodium vivax***, ***P falciparum***, ***P malariae***, and ***P ovale***. The two most common species are *P vivax* and *P falciparum*, with *falciparum* being the most pathogenic of all. *Plasmodium vivax*: 43% of total malarial cases in Iraq. Simple, benign, tertian.

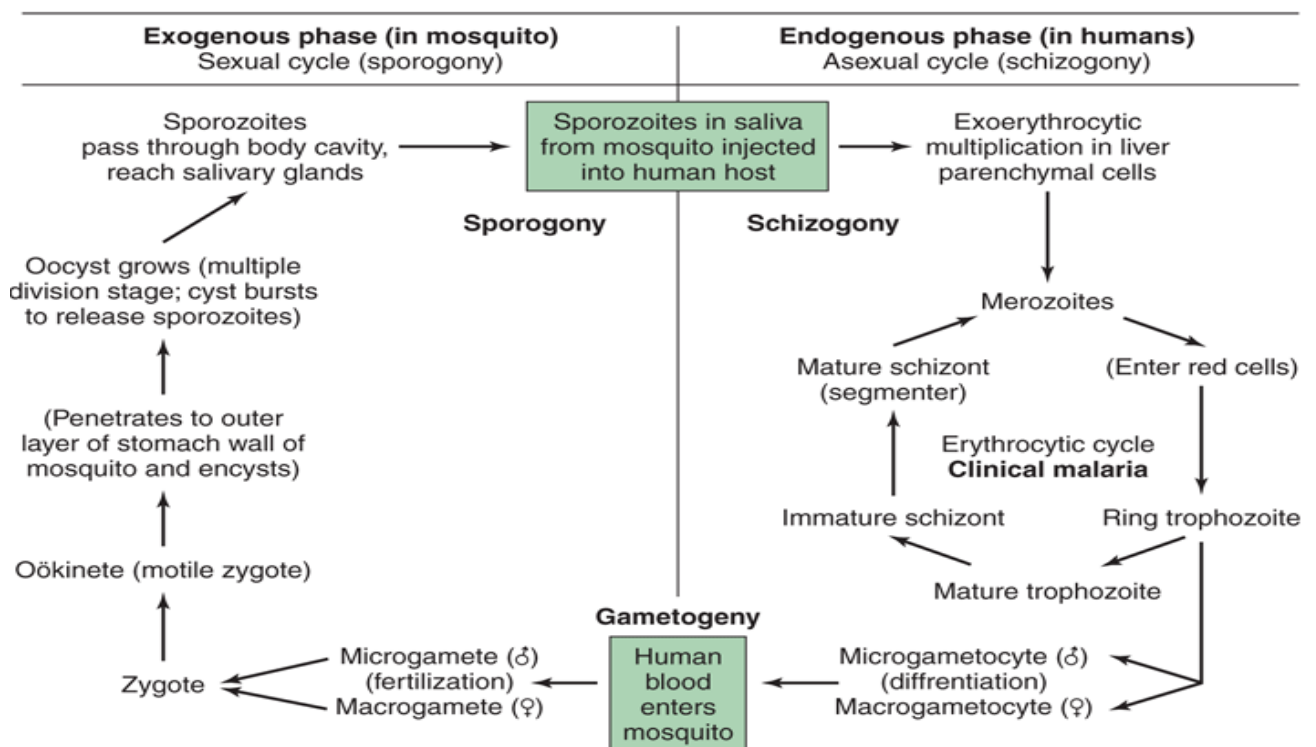
### **Life Cycle:**

#### **Human cycle**

**Exoerythrocytic stage -Schizogony:** Human infection results from the bite of infected female *Anopheles* mosquito, through which the sporozoites are injected into the bloodstream. The sporozoites rapidly (usually within 1 hour) enter parenchymal cells of the liver, where the first stage of development in humans takes place. Subsequently, numerous asexual progeny, the merozoites, rupture and leave the liver cells, enter the bloodstream, and invade erythrocytes. The merozoites do not return from red blood cells to liver cells.

**Erythrocytic cycle:** merozoites in the red cells multiply in a species-characteristic fashion, breaking out of their host cells synchronously, with successive broods of merozoites appearing at 48-hour intervals (*P vivax*, *P falciparum*, and *P ovale*) or every 72 hours (*P malariae*). The digestion of red cell hemoglobin, which is transformed into malaria pigment.

**Gametogony:** During the erythrocytic cycles, certain merozoites enter red cells and become differentiated as male or female gametocytes.



## Mosquito cycle

**Sporogony:** The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal (only mature sexual forms are capable of further development and rest die). In the mosquito's stomach, from one microgametocyte eight microgametes are formed by the process called exflagellation. The macrogametocyte does not show exflagellation, it develops into a macrogamete.

**Fertilization** occurs when the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated (ookinetes)

which invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release millions of spindle-shaped **sporozoites**, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into cutaneous blood vessels and initiates infection.

*P vivax* and *P ovale* may persist as dormant forms, or hypnozoites, after the parasites have disappeared from the peripheral blood. Resurgence of an erythrocytic infection (relapse) occurs when merozoites from hypnozoites in the liver break out, are not phagocytosed in the bloodstream, and succeed in reestablishing a red cell infection (clinical malaria). Without treatment, *P vivax* and *P ovale* infections may persist as periodic relapses for up to 5 years. *P malariae* infections lasting 40 years have been reported.

	<i>Plasmodium vivax</i> (Benign Tertian Malaria)	<i>P falciparum</i> (Malignant Tertian Malaria)	<i>P malariae</i> (Quartan Malaria)	<i>P ovale</i> (Ovale Malaria)
<b>Parasitized red cells</b>	Enlarged, pale, (Schüffner dots). invades young red cells	Not enlarged. (Maurer's clefts). Invades all red cells regardless of age	Not enlarged. No stippling (except with special stains). Primarily invades older red cells	Enlarged, pale. Schüffner dots. Cells often oval, or crenated
<b>Level of usual maximum parasitemia</b>	Up to 30,000/ $\mu$ L of blood	May exceed 200,000/ $\mu$ L; commonly 50,000/ $\mu$ L	Fewer than 10,000/ $\mu$ L	Fewer than 10,000/ $\mu$ L
<b>Ring stage trophozoites</b>	Large rings (1/3–1/2 red cell diameter). Usually one ring delicate	Small rings (1/5 red cell diameter). Often two ring delicate, may adhere to red cells	Large rings (1/3 red cell diameter). Usually one ring thick	Large rings (1/3 red cell diameter). Usually one ring thick
<b>Pigment in developing trophozoites</b>	Fine; light brown; scattered	Coarse; black; few clumps	Coarse; dark brown; scattered clumps; abundant	Coarse; dark yellow-brown; scattered
<b>Older trophozoites</b>	Very pleomorphic	Compact and rounded	Occasional band forms	Compact and rounded
<b>Mature schizonts (segmenters)</b>	More than 12 merozoites (14–24)	Usually more than 12 merozoites (8–32).	Fewer than 12 merozoites. Often in rosette	Fewer than 12 large merozoites. Often in rosette
<b>Gametocyte</b>	Round or oval	Crescentic	Round or oval	Round or oval
<b>distribution in peripheral blood</b>	All forms	Only rings and crescents (gametocytes)	All forms	All forms

### Some Characteristic Features of the Malarial Parasites of Humans

### **Pathogenicity and symptoms:**

1. Signs and symptoms are due to erythrocytic infection, not due to the Exo-erythrocytic.
2. Destruction of RBCs due to rupture method by parasite and lysis of non-parasitized cells due to debris and toxins metabolic byproducts of ruptured cells cause **anemia**.

The number of RBCs in severe cases equal to half of normal number. As the number of invaded RBCs increases and asexual cycles of parasite synchronized the quantity of pyrogen becomes sufficient to produce the characteristic chills and fever of malarial attack.

3. *P. vivax* and *P. ovale* infect youngest erythrocytes, while *P. malariae* infect oldest one.
4. *P. falciparum* invades erythrocytes of all ages, so produces extensive parasitemia and responsible for fatal infection.
5. In *P. falciparum* after 2-3 asexual cycles the number of infected RBCs reaches a dangerous threshold without production of typical chills and fever.
6. Erythrocytes containing *P. falciparum* adhere to one another and to lining of blood vessels causing blockage of blood capillaries in vital area such as brain, lung, and kidney.
7. Toxin production interfere with oxygen utilization by the host cells, and cause oxygen starvation of tissue followed by thrombosis in small blood vessels and decrease in the volume of circulating blood.
8. Spleen is typically enlarged and congested (**splenomegaly**), its color get darkened as amount of pigment increase and hard in chronic stage and soft and hemorrhagic in acute stage.
9. The liver hypertrophic and congested in acute malaria and contains deposits of pigments.
10. Bone marrow undergoes the same changes as spleen. Kidneys are congested.

11. Quarter malaria cause glomerulonephritis and pulmonary congestion.

**Malarial paroxysm:**

*P. vivax*, *P. ovale* or quarter malaria characterized by sudden develops with shaking chill followed by a fever of 40-40.6°C with headache, muscular pain, malaise, nausea, vomiting, and increase in pulse and respiration rates. After several hours, the fever terminates and followed by drenching sweat and patient will be exhausted. The series of paroxysm constitute the primary attack complication and pernicious symptoms: coma, convulsion, and cardiac failure.

**Diagnosis:**

-**Microscopy:** Thin and thick blood films taken just before or at the height of malarial paroxysm will detect number of parasites.

1. Thick blood film for rapid examination of a large volume of blood in small area on the slide (concentration of parasite).
2. Thin blood film for specific diagnosis of species.

-**immunochromatographic methods:** to detect the antigens in blood.

-other methods: as serology (detect antibodies) and PCR.

**Treatment:**

Treatment of malarial attacks: chloroquine and amodiaquine. Resistant malaria *P. falciparum* showing drug resistant, and treated with quinine alone or with combination with other drugs.

**Control:** requires community rather than individual reports.

1. Antimosquito chemicals: like **DDT (Dichloro Diphenyl Trichloroethane)** and Malathion are found to be effective to control by preventing the breeding of *Anopheles* mosquito.
2. Avoiding exposure to mosquito bites.
3. Taking chemoprophylaxis: Chloroquine and the antifolate drugs.

Worms of humans, belong to two phyla: Nematoda (roundworms) and Platyhelminthes (flatworms).

**Platyhelminthes** are flatworms that are dorsoventrally flattened in cross section and are hermaphroditic, with a few exceptions. All medically important species belong to two classes: **Trematoda** (flukes) and **Cestoda** (tapeworms).

**Cestodes**, or tapeworms, are flat and have a ribbon-like chain of segments (proglottids) containing male and female reproductive structures. Adult tapeworms can reach lengths of 10m and have hundreds of segments, with each segment releasing thousands of eggs. At the anterior end of an adult tapeworm is the scolex, which is often elaborated with muscular suckers, hooks, or structures that aid in its ability to attach to the intestinal wall. Behind the scolex there is a neck from which proglottids of the body are generated. Three types of proglottids are recognized; immature, mature and gravid. Adult tapeworms have no mouth or gut and absorb their nutrients directly from their host through their integument.

### ***Taenia saginata* & *T. solium***

#### Life cycle

Humans are the only definitive hosts for *Taenia saginata* (beef tapeworm) and *Taenia solium* (pork tapeworm). Natural habitat is the small intestine (upper jejunum) of man. Eggs or gravid proglottids are passed with feces; the eggs can survive for days to months in the environment. Cattle (*T. saginata*) and pigs (*T. solium*) become infected by ingesting vegetation contaminated with eggs or gravid proglottids.

In the animal's intestine, the oncospheres hatch, invade the intestinal wall, and migrate to the striated muscles, where they develop into cysticerci (cysticercus bovis in case of *T. saginata* and cysticercus cellulosae in case of *T. solium*). A cysticercus can survive for several

years in the animal. Humans become infected by ingesting raw or undercooked infected meat. In the human intestine, the cysticercus develops over 2 months into an adult tapeworm, which can survive for years. The adult tapeworms attach to the small intestine by their scolex and reside in the small intestine. Length of adult worms is usually 5 m or less for *T. saginata* (however it may reach up to 25 m) and 2 to 7 m for *T. solium*. The adults produce proglottids which mature, become gravid, detach from the tapeworm, and migrate to the anus or are passed in the stool (approximately 6 per day). *T. saginata* adults usually have 1,000 to 2,000 proglottids, while *T. solium* adults have an average of 1,000 proglottids. The eggs contained in the gravid proglottids are released after the proglottids are passed with the feces. *T. saginata* may produce up to 100,000 and *T. solium* may produce 50,000 eggs per proglottid respectively.

### **Pathology & Pathogenesis**

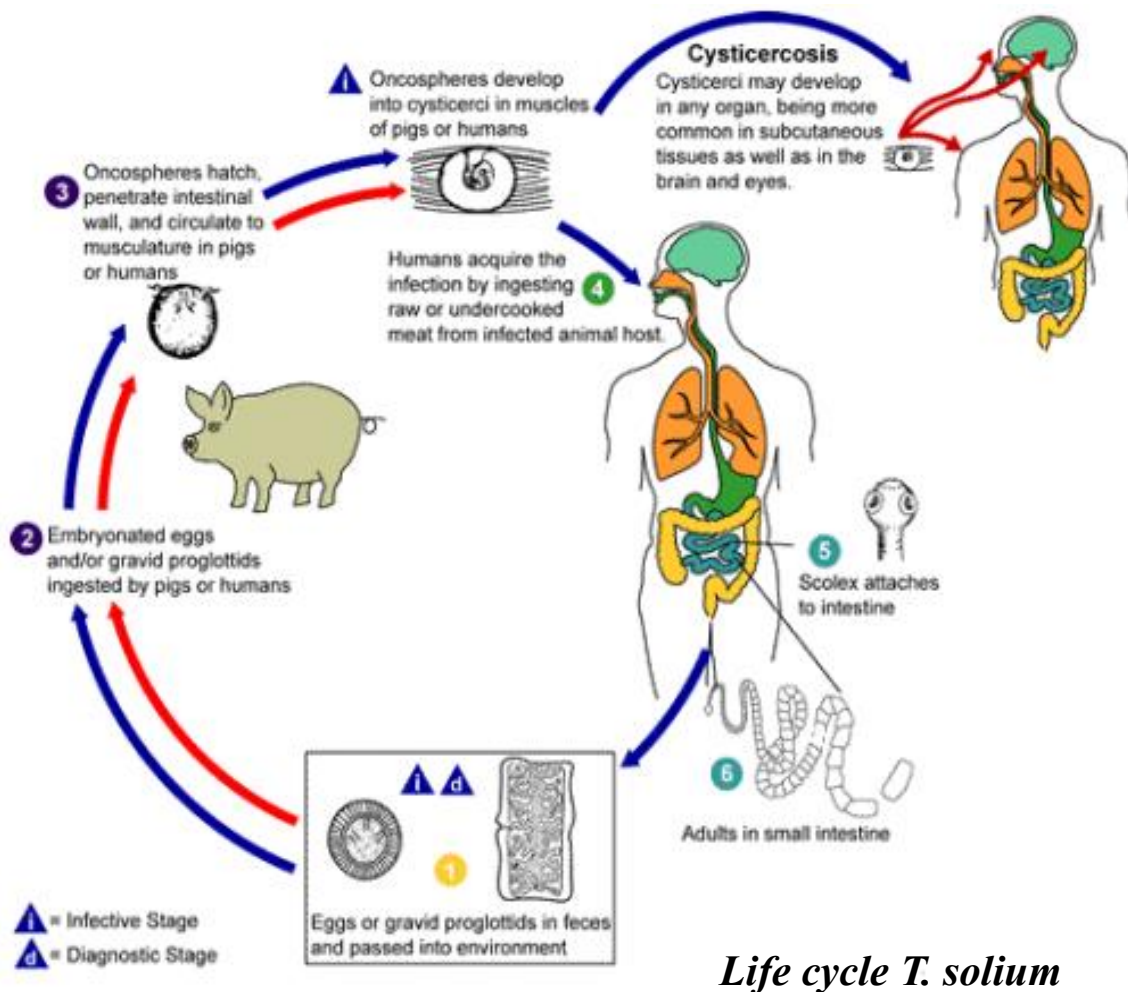
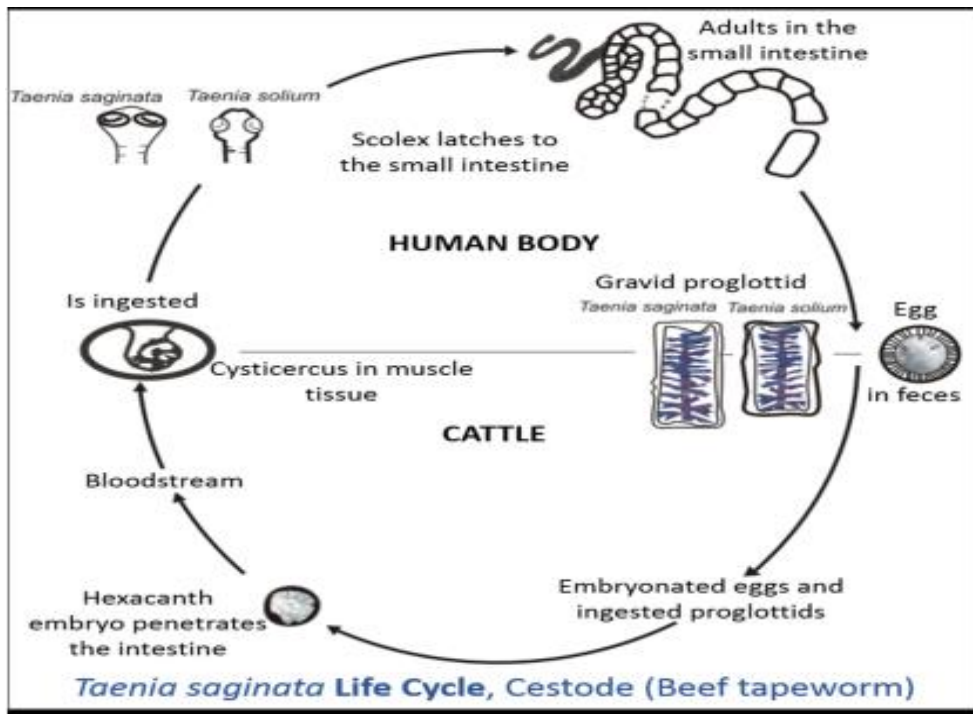
One medically significant difference between *T. saginata* and *T. solium* is that humans can be the intermediate host for *T. solium*, similar to pigs. Thus, if humans ingest *T. solium* eggs, the cysticerci encyst in various human tissues, including skin, muscle, kidney, heart, liver, and brain. This condition in humans is known as cysticercosis, and symptoms are associated with the involved tissues (eg, diminution of visual acuity with ophthalmocysticercosis; in neurocysticercosis, symptoms include headache, nausea, vomiting, mental disturbances, and seizures caused by encysted cysticerci in the brain). With the beef tapeworm *T. saginata*, adult worms develop only in humans, and cysticerci of *T. saginata* do not develop in humans (only in cattle or other herbivores).

### **Diagnosis**

The diagnosis of both tapeworms disease is made by finding eggs or proglottids in the stool. The adhesive cellophane tape technique described for pinworm can be used to recover the worms from this area. Because the eggs of *T. solium* and *T. saginata* are morphologically



identical, it is necessary to examine a proglottid to identify the species.





## **Treatment and Prevention**

The drugs of choice are praziquantel or niclosamide, which act directly on the worm. Both are highly effective in single-dose oral preparations. Ultimately, control is best effected through the sanitary disposal of human feces. Meat inspection is helpful; the cysticerci are readily visible. In areas where the infection is common, thorough cooking is the most practical method of control. Internal temperatures of 56°C or more for 5 minutes or longer destroy the cysticerci. Salting or freezing for 1 week at -15°C or less is also effective.

### ***Hymenolepis nana* (Dwarf Tapeworm—Intestinal Cestode)**

*Hymenolepis nana*, the dwarf tapeworm (only about 4 cm in length) of humans (and rodents). The incidence is higher in children. It is found worldwide and is one of the most common tapeworm infections in humans.

### **Life cycle**

In humans the adult tapeworms are found in the upper two thirds of ileum, whereas in rodents they are found in posterior part of ileum.

-Direct cycle: the eggs are passed in the faeces of infected man or rodent. Man acquire infection by ingestion of contaminated food or water. In the intestine a free oncosphere is liberated from the egg. It penetrates into a villus and develop into cysticercoid larva. Later, it attaches to the intestine wall and develop to adult. In heavy infections the eggs may hatch in the intestine before passing out in faeces, resulting in autoinfection.

-Indirect cycle: in presence of insects (include beetles) as the intermediate host. These insects eat eggs of *H. nana*. In the body cavity of insect, the oncosphere transform into cysticercoid, which is infective to final host. Man is infected by accidentally ingestion of these insects. In the intestine the cysticercoid larva develop into adult worm.

## Pathogenesis

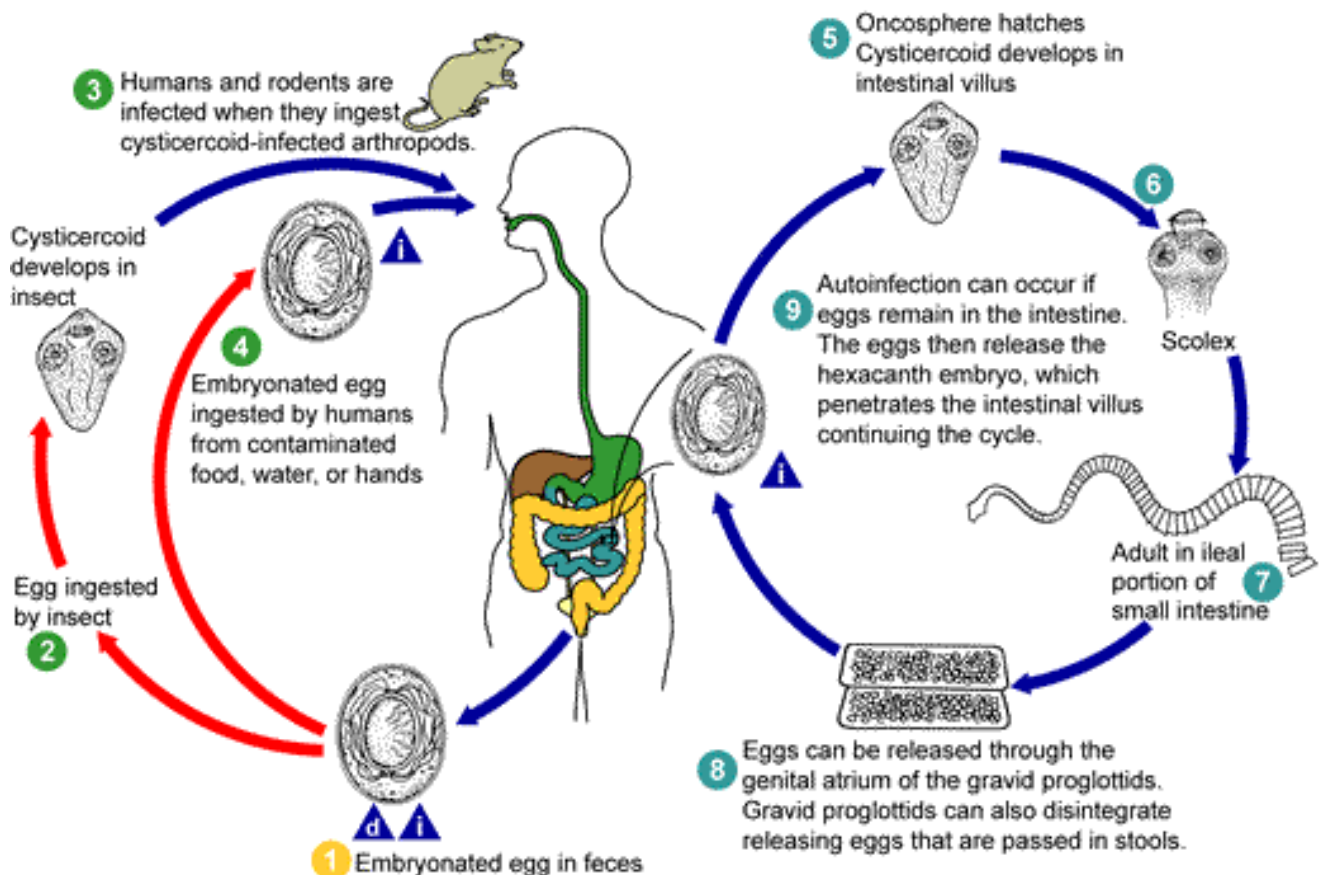
The mechanism by which symptoms are produced is an allergic reaction. Patient develops headache, anorexia, abdominal pain, diarrhea and eosinophilia.

## Diagnosis

The diagnosis is made by finding eggs in the stool by direct microscopy.

## Prevention and treatment

Preventing fecal contamination of food and water in institutions and crowded areas is of primary importance. Rodent and insect control (especially control of fleas and grain insects) are also essential for prevention. The drug of choice is praziquantel and the second choice is niclosamide.



Life cycle of *Hymenolepis nana*

**Leishmania spp.**

*Leishmania* spp. caused Leishmaniasis disease spread by the bite of certain types of sandflies. Geographical distribution divided the disease to 1- Old world leishmaniasis caused by *L. donovani*, *L. infantum*, *L. tropica*, *L. major*, *L. aethiopica* are transmitted by the sandflies genus *Phlebotomus*; 2- New world leishmaniasis caused by *L. Mexicana*, *L. braziliensis* and etc... are transmitted by the sandflies genus *Lutzomyia*.

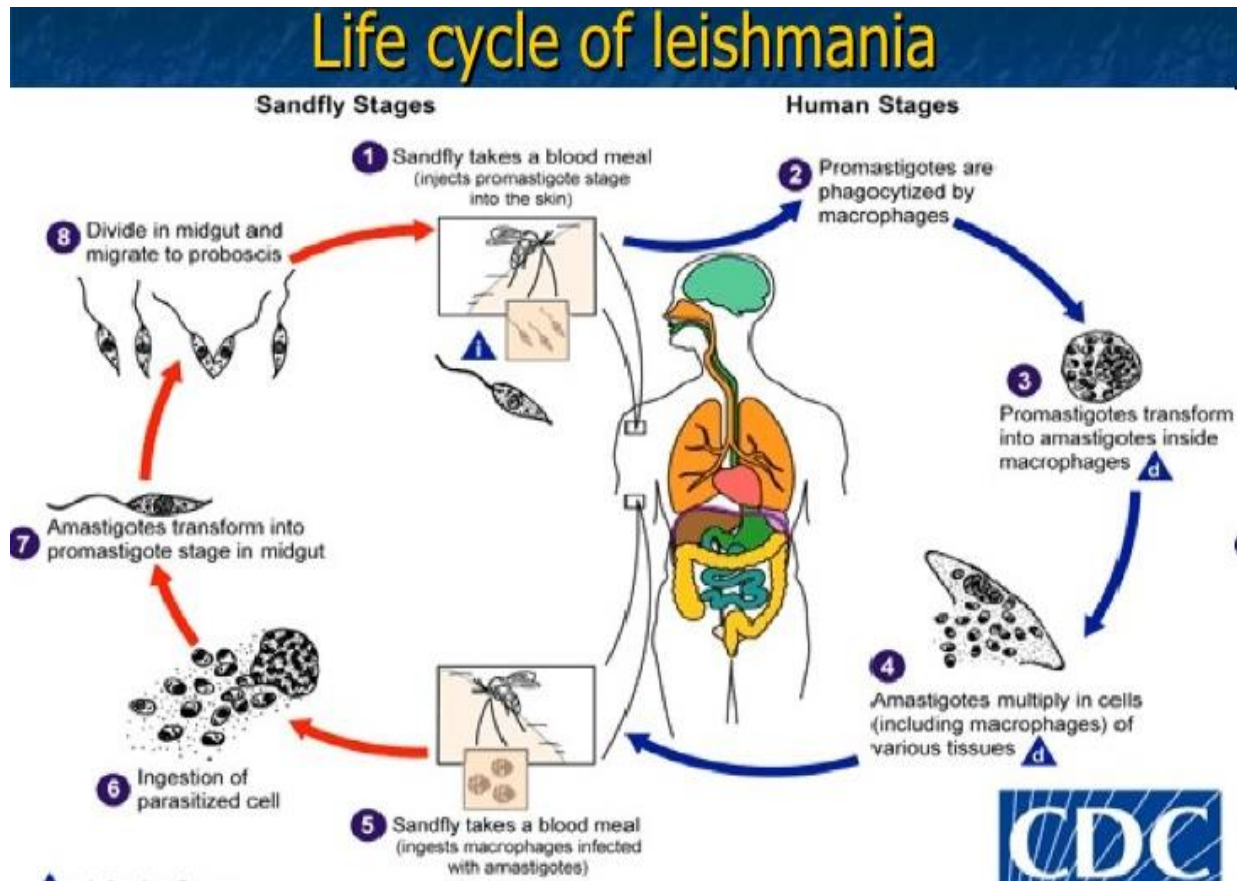
Risk factors include poverty, malnutrition, deforestation, lack of sanitation and urbanization.

**Morphology and Life cycle**

The life cycle of *Leishmania* is completed in two hosts, humans and sandflies. Two stages are known; the amastigote which is spherical or subspherical and the promastigote which is pyriform or spindle shape with flagellum. Natural reservoir hosts humans, dogs and wild rodents. The adult female sandfly is a bloodsucker, usually feeding at night. When the fly bites a reservoir host or infected person with *Leishmania*, the pathogen reaches the stomach of the sandfly, the amastigotes quickly transform into elongated and motile forms called the promastigotes. The promastigotes live extracellularly in the alimentary canal, reproducing asexually, then migrate to the proximal end of the gut. As the fly bites, the promastigotes are released from the proboscis and introduced locally at the bite site.

Once inside the human host, promastigotes invade macrophages. Inside the cells they transform back into the smaller amastigote form. The amastigotes replicate in the macrophage cell. After repeated multiplication, they break down their host cell by utter pressure of mass. The daughter cells protozoans then migrate to fresh cells (Cutaneous or mucocutaneous leishmaniasis) or through the bloodstream

(visceral leishmaniasis) to find new hosts. In this way the infection is progressive, spreading to the host's mononuclear phagocyte system, particularly the spleen and liver. The free amastigotes in peripheral tissues are then ingested by sandfly to enter another cycle.



## Old world leishmaniasis

- **Cutaneous leishmaniasis (oriental sore)**

Is the most common form caused by *L. tropica*, *L. major*, *L. aethiopica*, found in 88 tropical and subtropical countries which causes an open sore at the bite sites, which heals in a few months to a year and half, leaving an unpleasant-looking scar. Diffuse cutaneous leishmaniasis produces widespread skin lesions which resemble leprosy, and may not heal on its own.

**Pathogenesis:** *Leishmania* invades human macrophages and replicates intracellularly. A raised, red lesion develops at the site of the bite (often weeks or sometimes years afterwards). The lesion then ulcerates and may become secondarily infected with bacteria.

### **Diagnosis**

-A skin scraping with microscopic analysis using Wright or Giemsa stain is the best test.

-Needle aspiration of tissue fluid from the margin of a lesion can yield fluid for culture to isolate the organism and identify the species.

-DNA testing (PCR).

-Leishmanin skin test (Montenegro test) delayed-type hypersensitivity reaction.

#### **- Visceral leishmaniasis (kala-azar)**

Also known as black fever, and Dumdum fever, is the most severe form of leishmaniasis and, without proper diagnosis and treatment, is associated with high fatality. Caused by obligate intracellular parasite *Leishmania donovani*, *L. infantum* (infantile visceral leishmaniasis) Habitat: reticuloendothelial cells, predominately of liver, spleen, bone marrow and lymph nodes of man and dogs.

### **Pathogenesis:**

The parasite spreads from the site of inoculation to multiply in reticuloendothelial cells, especially in the liver, spleen, bone marrow and lymph nodes. This leads to progressive enlargement of these organs. The most typical symptoms are fever and the enlargement of the spleen, liver and lymph nodes, anaemia, leucopenia, and skin changes. Death is due to secondary infections.

Sometime after successful treatment—generally a few months with African kala-azar, or as much as several years with the Indian strain—a secondary form of the disease may set in, called post kala-azar dermal leishmaniasis, or PKDL. This condition caused by the reversal of *L.donovani* from viscerotropic to dermatotropic, manifests first as small, measles-like skin lesions on the face, which gradually increase in size and spread over the body.

### **Diagnosis**

**Non specific tests:** blood count, haemoglobin ad serum protein estimation.

**Parasitological diagnosis:** blood film stained with Leishman or Giemsa stain, culture in specific media, visualization of the amastigotes in splenic or bone marrow aspirate, PCR (polymerase chain reaction) tests for the detection of Leishmania DNA.

**Immunological tests:** Serological testing is much more frequently used in areas where leishmaniasis is endemic.

### **New world leishmaniasis**

#### **- Mucocutaneous leishmaniasis (espondia)**

It causes both skin and mucosal ulcers with damage primarily of the nose and mouth. It can be highly disfiguring if not promptly treated. Caused by *L. Mexicana*, *L. braziliensis*, which are mainly found in certain South American nations. Severe cases can diminish the ability to eat and can be fatal.

**Pathogenesis:** *Leishmania* invades human macrophages and replicates intracellularly. A raised, red lesion develops at the site of the bite. The lesion may spontaneously heal with scarring, but then reappear elsewhere (especially as destructive mucocutaneous lesions).

**Diagnosis:** as in cutaneous leishmaniasis

**Treatments:**

The treatment needed is determined by where the disease is acquired, the species of Leishmania, and the type of infection. Some possible medications used for visceral disease include liposomal amphotericin B, a combination of pentavalent antimonials and paromomycin, and miltefosine. For cutaneous disease, paromomycin, fluconazole, or pentamidine may be effective.

**Prevention**

Leishmaniasis can be partly prevented by:

- sleeping under nets treated with insecticide.
- spraying insecticides to kill sandflies.
- treating people with the disease early to prevent further spread.
- reservoir control programs.



**Parasitology** is the area of biology concerned with the phenomenon of dependence of one living organism on another. Medical parasitology deals with the parasites which infect man, the diseases they produce, the response generated by him against them, and various methods of diagnosis, prevention and treatment.

**Parasite:** is an organism that is entirely dependent on another organism, referred to as its host, for all or part of its life cycle and metabolic requirements. Strictly speaking, the term parasite can be applied to any infectious agent but, by convention, it is generally restricted to infections caused by protozoa and helminths and excludes the viruses, bacteria and fungi.

Parasite is of two types:

**Microparasite:** small, unicellular and multiplies within its vertebrate host, often inside cells. Protozoa are microparasites.

**Macroparasite:** large, multicellular and has not direct reproduction within its vertebrate host. This category include helminths.

On the basis of their location, parasites may be divided into:

**Ectoparasites:** which live on the surface of the body, e.g., the human louse, *Pediculus humanus*. The infection by these parasites is known as infestation. They are important as vectors transmitting pathogenic microorganisms.

**Endoparasites:** which live within the body of the host. All protozoan and helminthic parasites of man are endoparasites. The invasion by endoparasites is known as infection. These can be further subdivided into following types:

**Obligate parasites:** organisms cannot exist without a host e.g. *Toxoplasma gondii*.

**Facultative parasites:** organisms that under unfavorable circumstances may live either a parasitic or free- living existence e.g. *Naegleria fowleri*.

**Accidental parasites:** organisms that attack an unusual host e.g. *Echinococcus granulosus* in man.

**Aberrant parasites:** organisms that attack a host where they cannot live or develop further e.g. *Toxocara canis* in man.

**Free- living:** the term free-living describes the nonparasitic stages of existence which are live independently of a host e.g. hookworms have active free-living stages in the soil.

\*Why a human embryo or fetus is not a parasite?

**Host:** organism which harbors the parasite and provides the nourishment and shelter. It is of following types:

**Definitive host:** harbors the adult parasite, where the parasite replicates sexually.

**Intermediate host:** the host which alternates with the definitive host and harbors the larval or asexual stages of a parasite. Some parasites require 2 intermediate hosts for completion of their life cycle.

**Paratenic host:** a host in which larval stage of a parasite survives but does not develop further. It is often not a necessary part of life cycle.

**Reservoir host:** can harbor a pathogen indefinitely with no ill effects. Once discovered, natural reservoirs elucidate the complete life cycle of infectious diseases, providing effective prevention and control.

**Compromised host:** one in whom normal defence mechanisms are impaired e.g. AIDS. Such hosts are extremely susceptible to a variety of pathogens.

### **Host- parasite relationships:**

**Symbiosis:** an association in which both host and parasite are so dependent upon each other that one cannot live without the help of the other. Neither of the partners suffers from any harm from this association.

**Commensalism:** an association in which only parasite may benefit without detectable damage to the host as in case of *Entamoeba coli* in the large intestine of man, (One partner benefits but the other is not hurt).

**Parasitism:** One partner (the parasite) harms or lives on the expense of the other (host). The degree of dependence of a parasite on its host varies.

### **Classification of animal parasite and vectors:**

- Phylum
  - Subphylum
    - Class
      - Order
        - Family
          - Genus
            - Species

All of these names must be of Greek or Latin origin or have a classical termination.

**Species:** it designates a population, the members of which have essentially the same genetic characters and are capable of continued reproduction of their kind, but usually cannot interbreed with individuals of other species.

**Genus:** is a group of closely related species.

The scientific designation of a species is a combination of the genus and species name. This is referred to as binomial nomenclature. Ex. *Entamoeba histolytica*.

**Classification of the protozoa:**

Human parasites in the kingdom Protista, subkingdom Protozoa are classified under four phyla:

- 1- Sarcomastigophora (containing amoeba and flagellates)
- 2- Apicomplexa (containing Sporozoa)
- 3- Ciliophora (containing Ciliates)
- 4- Microspora

**1- Sarcomastigophora**

This phylum is subdivided into two subphyla:

- 1- Sarcodina
- 2- Mastigophora

**Sarcodina:** amoeboid organisms using pseudopodia for both locomotion and feeding. Examples:

- Entamoeba histolytica*
- E. coli*
- E. gingivalis*
- Iodamoeba buetschlii*
- Endolimax nana*

**Vector:** an agent, usually an insect that transmits infection from one human host to another. Mechanical vector term used to describe a vector which assists in transfer parasitic forms between hosts but is not essential in the life cycle of the parasite, e.g. a housefly that transfers amoebic cysts from infected faeces to food that is eaten by humans.

**Zoonosis:** term describe a disease communicable from animals to humans (enzootic infection acquired by man) under natural conditions. Examples include; leishmaniasis, hydatid cyst and fascioliasis.

Definition of sporulation:

The formation of a single refractory body, or resting spore, within certain bacteria (such as *Bacillus* and *Clostridium*) that makes the cell resistant to unfavorable environmental conditions. The cell regains its viability when conditions become favorable. Bacterial spores are endospores in contrast to fungal spores, which are usually exospores. Unlike the spores of fungi, bacterial spores do not serve reproductive function.

Mechanism of sporulation

The formation of a spore is a complex process for the bacterial cell. Spores are only made under conditions where cell survival is threatened such as starvation for certain nutrients (especially the lack of carbon and nitrogen sources) or accumulation of toxic wastes. Regulation of sporulation is tight and the first few steps are reversible. This helps the cell conserve energy and only sporulate when necessary. The genetic basis for the obligation to form a spore is a protein called SpoA. This protein functions to promote the transcription of genes that are required for the conversion of the actively growing bacterium to a spore.

Sporulation is a seven step process, which takes about eight hours.

**Stage 1:** involve the forming of a separate compartment for the spore in the mother cell and the DNA is replicated.

**Stage 2:** a membrane wall known as a spore septum begins to form between it and the rest of the cell. The plasma membrane of the cell surrounds this wall and pinches off to leave a double membrane around

the DNA, and the developing structure is now known as a forespore. Once this occurs, sporulation is irreversible.

**Stage 3:** the mother cell membrane continues to grow and engulfs the developing spore.

**Stage 4:** the peptidoglycan cortex forms between the two layers. The cortex contains an inner membrane known as the core. The inner membrane that surrounds this core leads to the endospore's resistance against UV light and harsh chemicals that would normally destroy microbes. The cortex is what makes the endospore so resistant to temperature.

**Stage 5:** the bacterium adds a spore coat to the outside of the forespore. Calcium dipicolinate is incorporated into the forespore during this time. The dipicolinic acid helps stabilize the proteins and DNA in the endospore. Both the spore and the mother cell plays a role in this process.

**Stage 6:** The spore dehydrates its cytoplasm (osmotically as  $\text{Ca}^{++}$  enters, the water is removed) and the spore become mature. Mature spore form additional layer called exosporium.

**Stage 7:** the mature spore will be released when the surrounding vegetative mother cell is degraded by lytic enzymes.

**Reactivation** of the endospore occurs when conditions are more favorable and involves *activation*, *germination*, and *outgrowth*.

Activation: Even if an endospore is located in plentiful nutrients, it may fail to germinate unless activation has taken place. This may be triggered by heating the endospore.

Germination: involves the dormant endospore starting metabolic activity and thus breaking hibernation. It is commonly characterized by rupture or absorption of the spore coat, swelling of the endospore, an increase in metabolic activity, and loss of resistance to environmental stress.

Outgrowth: follows germination and involves the core of the endospore manufacturing new chemical components and exiting the old spore coat to develop into a fully functional vegetative bacterial cell, which can divide to produce more cells.

Certain types of endospores are used to ensure that an autoclaved item has been rendered truly sterile: a small capsule containing the spores is put into the autoclave with the items; after the cycle it is checked to see if anything will grow from it. If nothing will grow, then the spores were destroyed and the sterilization was successful.

In hospitals, endospores on delicate invasive instruments (e.g., video endoscopes) are killed by low-temperature and non-corrosive, non-toxic, plasma-activated concentrated hydrogen peroxide vapor in sterilizers. In contrast, "high level disinfection" does not kill endospores but is used for instruments that don't enter sterile bodily cavities (e.g., a colonoscope). This latter method uses only warm water, enzymes, and detergents.

Bacterial endospores are resistant to antibiotics, most disinfectants, and physical agents such as radiation, boiling, and drying. Prolonged exposure to ionising radiation, such as x-rays and gamma rays, will kill most endospores.



The heat resistance of endospores is due to a variety of factors:

Small acid-soluble proteins (SASPs) saturate the endospore's DNA and protect it from heat, drying, chemicals, and radiation. They also function as a carbon and energy source for the development of a vegetative bacterium during germination.

Finally, DNA repair enzymes contained within the endospore are able to repair damaged DNA during germination.

Endospores possess five times more sulfur than vegetative cells. This excess sulfur is concentrated in spore coats as an amino acid, cystine. It is believed that the macromolecule accountable for maintaining the dormant state has a protein coat rich in cystine, stabilized by S-S linkages. A reduction in these linkages has the potential to change the tertiary structure, causing the protein to unfold. This conformational change in the protein is thought to be responsible for exposing active enzymatic sites necessary for endospore germination.

### **Parts of the Spore**

- **Core** - The core is dehydrated cytoplasm containing DNA, ribosomes, enzymes etc. Everything that is needed to function once returned to the vegetative state.
- **Cortex** - The cortex is a modified cell wall/peptidoglycan layer that is not as cross-linked as in a vegetative cell.
- **Coats** - Outside of the cortex are several protein layers that are impermeable to most chemicals. The coat is responsible for the resistance to chemicals.

## Properties:

1. Endospores can stay dormant for a very long time (many years or centuries).
2. They can tolerate extreme dryness, heat and radiation
3. Some cannot be killed even at subzero temperatures.
4. Most disinfectants such as household cleaning products, alcohols, quaternary ammonium compounds and detergents have little effect on endospores.
5. Can be destroyed by burning or by autoclaving at a temperature exceeding the boiling point of water, 100 °C. Endospores are able to survive at 100 °C for hours, although the longer the number of hours the fewer that will survive. Or destroyed by Tyndallization (Duty).
6. Not stained readily with dyes.
7. No DNA replication (transcription).
8. No mRNA production (translation).
9. Protein synthesis is defective.

The **position** of spores differs among bacterial species and it is useful in identification, spores may be:

1. Central or equatorial, giving the bacillus a spindle shape (eg. *Clostridium bifermentans*)
2. Subterminal, the bacillus appearing Club shaped (eg. *Clostridium perfringens*)

3. Oval and terminal, resembling a tennis racket (eg. *Clostridium tertium*)
4. Spherical and terminal, giving a drumstick appearance (*Clostridium tetani*)

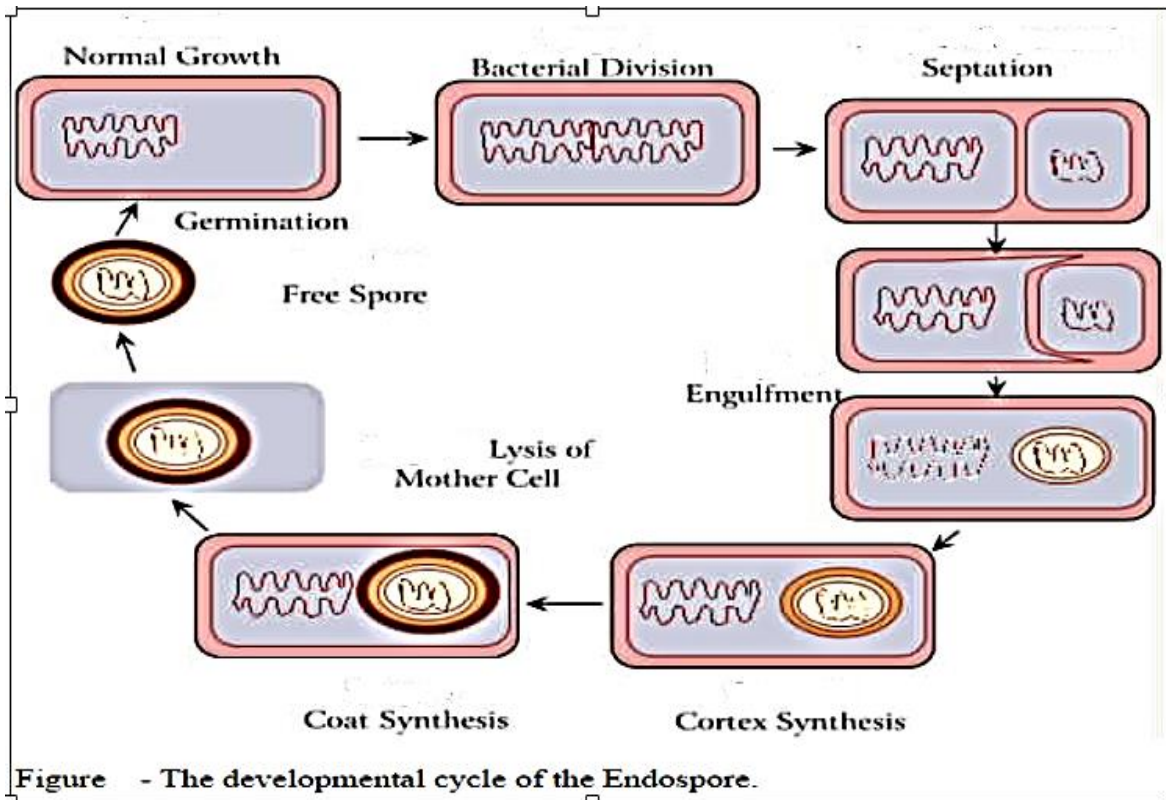


Figure - The developmental cycle of the Endospore.

**LEC.2                      Protozoa (Amoebae)                      Dr. Maysoon A.Merdaw**

Protozoa are single-celled animals; each cell performs all of the necessary functions of life, majority of which are free-living.

Amoebae belong to phylum Sarcomastigophora, subphylum Sarcodina.

Only *Entamoeba histolytica* is of medical importance.

***Entamoeba histolytica***

Morphology: the parasite exists in three forms; trophozoite, precyst and cyst. Trophozoites; 10-60 µm size, unidirectional motility, single pseudopodium, ingest erythrocytes, granular cytoplasm small, central karyosome, and beaded chromatin.

Habitat: trophozoites reside in mucosa and submucosa of large intestine of man.

Life cycle: Infection by *E. histolytica* occurs by ingestion of mature cysts (quadrinucleate) in faecally contaminated food, water, or hands. Excystation occurs in the small intestine and trophozoites are released, which migrate to the large intestine. The trophozoites multiply by binary fission and produce cysts, and both stages are passed in the feces. Because of the protection conferred by their walls, the cysts can survive days to weeks in the external environment and are responsible for transmission.

Trophozoites passed in the stool are rapidly destroyed once outside the body, and if ingested would not survive exposure to the gastric environment. In many cases, the trophozoites remain confined to the intestinal lumen (noninvasive infection) of individuals who are asymptomatic carriers, passing cysts in their stool. In some patients the trophozoites invade the intestinal mucosa and through the bloodstream can reach extraintestinal sites with resultant pathologic manifestations. It has been established that the invasive and noninvasive forms represent two separate species, respectively *E. histolytica* and *E. dispar*.

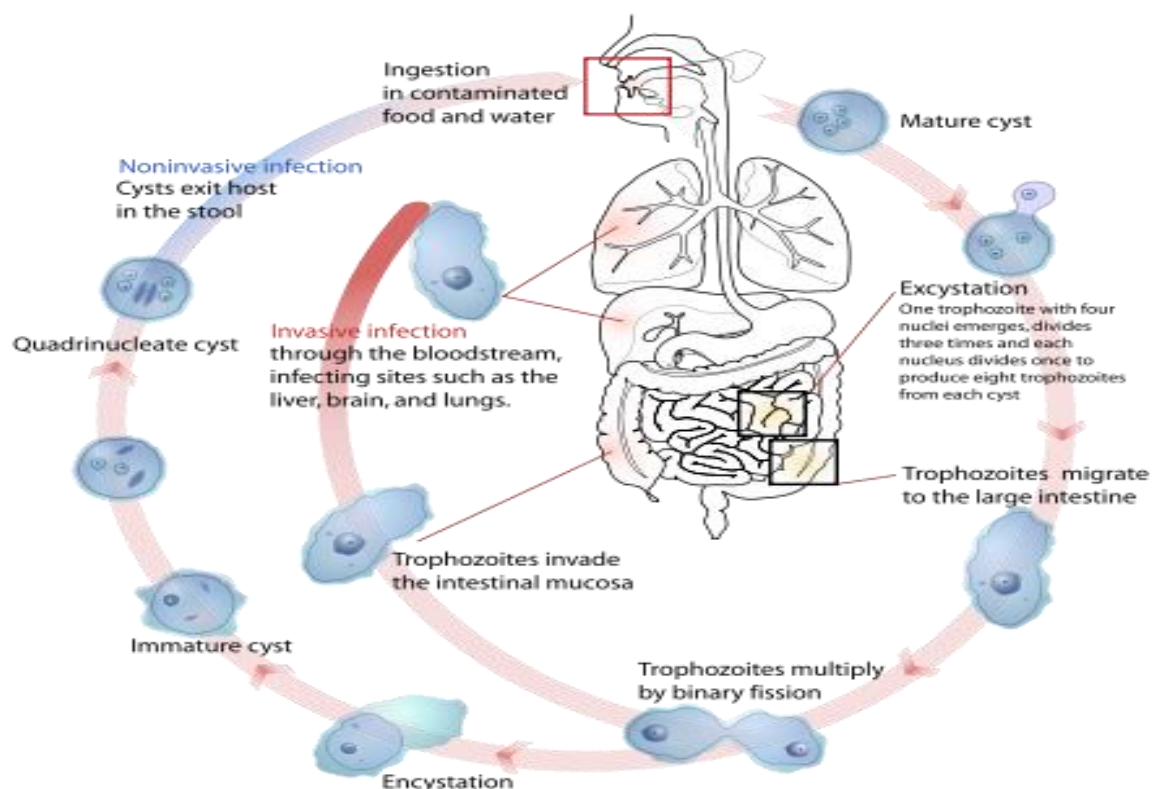
These two species are morphologically indistinguishable unless *E. histolytica* is observed with ingested red blood cells (erythrophagocytosis).

**Encystation:** occurs in the intestinal lumen in which chromatin materials are concentrated into bars, or grape like clusters (chromatoidal bodies) in the cytoplasm of the cyst.

The nucleus of cyst divides into two, then each of the two daughter nuclei divided once again so mature cyst has four nuclei, diffuse glycogen become concentrated in a mass with a hazy margins.

Viable cyst in the external environment are soon killed by drying, bacterial putrefactions of the medium, hypertonicity, direct sunlight and heat.

**Excystation:** occurs only after mature cyst has been taken in suitable host and reach a suitable level of intestine so amoeba becomes active, rupture the cyst membrane and escapes and started to multiply by binary fission and colonization inside host digestive tract.



**Pathogenesis:**

Intestinal amoebiasis: After an incubation period of 1-4 weeks, the amoeba invade colonic mucosa. During growth, *E. histolytica* secretes a proteolytic enzymes, producing flask- shaped ulcers and profuse bloody diarrhea (amoebic dysentery). Ulcers may be deep or superficial.

*E. histolytica* may also cause amoebic appendicitis and amoebomas (pseudotumoral lesions associated with necrosis, inflammation and oedema).

Extraintestinal amoebiasis: About 5-10% individuals with intestinal amoebiasis, 1-3 months after disappearance of dysentery, develop hepatic amoebiasis. Trophozoites are carried from the ulcer in the large intestine and multiply in the liver, lead to cytolytic action then small abscesses merge to form big liver abscesses. The abscesses may grow in various directions; it may enter into general circulation involving lungs, brain, spleen, skin, etc.

**Pathogenicity depend on:**

1. Virulence of strain
2. Resistance of the host (depends on the innate immunity).
3. State of nutrition of the host.
4. Infection with other agents (free of other infections mean less susceptible to infection).
5. Some drugs may irritate the intestinal wall so irritated intestine is more susceptible to infection.
6. Bacterial flora (metabolic processes can enhance the invasiveness).

**Symptoms:**

Fever, chills, and diarrhea, sometimes bloody or white mucus and often with cramps. Some people may have only mild abdominal discomfort

or no symptoms at all. Symptoms can start 2 or more weeks after infection.

### **Laboratory Diagnosis:**

*Entamoeba histolytica* must be differentiated from other intestinal nonpathogenic amoebae. The nonpathogenic *Entamoeba dispar* is morphologically identical to *E. histolytica*, and differentiation must be based on isoenzymatic or immunologic analysis. Molecular methods are also useful in distinguishing between *E. histolytica* and *E. dispar*.

-Microscopic identification of cysts and trophozoites in the stool is the common method for diagnosing *E. histolytica*. This can be accomplished using wet mount and permanently stained preparations as Iodine or trichrome or by Flotation or Sedimentation method for stool samples.

-Blood examination: shows moderate leukocytosis.

-Serological tests: in later stages of invasive amoebiasis, antibodies appear. Tests include ELISA, IHA and IFA.

-Histology: trophozoites can be identified in aspirates or biopsy samples obtained during colonoscopy or surgery.

- Molecular methods: DNA probe and PCR.

### **Treatment:**

For asymptomatic infections, paromomycin or iodoquinol are the drugs of choice. For symptomatic intestinal or extraintestinal infections, the drugs of choice are metronidazole or tinidazole, immediately followed by treatment with paromomycin or iodoquinol. Failure of metronidazole therapy may be an indication for surgical intervention.

### **Nonpathogenic amoebae:**

Several species of protozoans may be mistaken for *E. histolytica*. Care must be taken to correctly identify the infection.



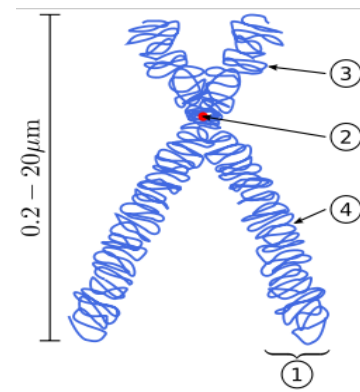
<i>Entamoeba dispar</i>	Morphologically identical to <i>E. histolytica</i> . It must be separated by isoenzymatic, immunologic or molecular analyses.
<i>Entamoeba hartmanni</i>	Some consider this a separate species. It differs from <i>E. histolytica</i> by being smaller in size.
<i>Entamoeba coli</i>	Distinguished from <i>E. histolytica</i> by having an eccentric endosome, and mature cysts with 8 nuclei. If chromatoidal bodies are present, they have splintered ends, rather than rounded as in <i>E. histolytica</i> .
<i>Endolimax nana</i>	This is a very small amoeba (6-15um) with a large, eccentric endosome and thin nuclear envelope. Mature cysts contain 4 nuclei.
<i>Iodamoeba butschlii</i>	Both the trophozoite and cyst have one nucleus with a large endosome. The cyst contains a large glycogen vacuole that stains darkly with iodine.

### Opportunistic amoebae

***Naegleria fowleri*:** It is a free-living amoeba that can be pathogenic, causing a fulminant brain infection called naegleriasis. This microorganism is typically found in warm freshwater, such as ponds, lakes, rivers, and hot springs. It is also found in the soil near warm-water discharges of industrial plants, and in unchlorinated or minimally-chlorinated swimming pools. It can be seen in either an amoeboid or temporary flagellate stage. *N. fowleri* is inhaled through the nose, where it then enters the nasal and olfactory nerve tissue, travelling to the brain. *N. fowleri* normally eat bacteria, but when it enters humans, it uses the brain as a food source. It does not form a cyst in human tissue, where only the amoeboid trophozoite stage exists. The flagellate form can exist in the cerebrospinal fluid. Death will usually occur within 1–2 weeks.

**Chromosome:** is a packaged and organized structure containing most of the DNA of a living organism. It is not usually found on its own, but rather is complexes with many structural proteins called histones. Two "sister" chromatids (half a chromosome) join together at a protein junction called a centromere. Chromosomes are normally visible under a light microscope only when the cell is undergoing mitosis.

- 1- chromatid
- 2- centromere
- 3- Short arm ( P arm)
- 4- Long arm ( Q arm)



**Bacterial chromosome:** consist of a circular double stranded DNA molecule, since prokaryotes do not have a nuclear membrane, each bacterial chromosome is packed in the structure called the nucleoid. DNA packing is achieved through coiling, compacting and supercoiling through twisting forces and formation of looped structure. Most prokaryotes are haploid (1n: one set of chromosomes), therefore they have one copy of each gene.

Some prokaryotes also have plasmids, which are small, circular DNA molecules that often carry genes responsible for characteristics like fertility, antibiotic resistance.

Plasmids are not a part of nucleoid, they can be copied and transmitted between cells or incorporated into chromosomal DNA and reproduced during cell division.

\*Is a human being has a plasmid?

### **DNA (Deoxyribose Nucleic Acid):**

Made up of 4 nucleotide, each one composed of; one of the 4 nitrogen bases (Adenine, Thymine, Guanine, and Cytosine) and a negatively

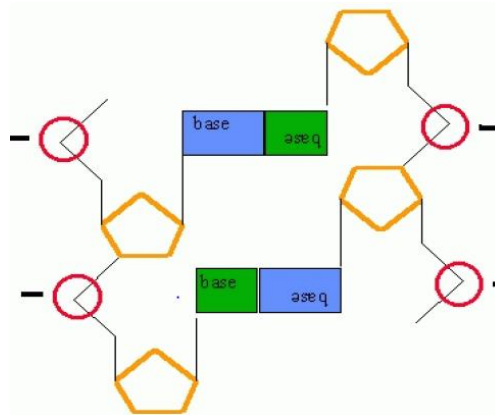
charged sugar-phosphate backbone. The sequence of these nucleotides is the genetic information organisms inherit. These bases align in two antiparallel strands where they pair with the complementary base (A bonds with T and G bonds with C) and give DNA its double helix shape. Hydrogen bonds link each complementary base pair. A=T, G=C. Glycoside bond link each nucleotide to the ribose sugar.

DNA is antiparallel because its carbons are aligned in 5' end (PO<sub>4</sub> group) to 3' end (OH<sup>-</sup> group) for coding and replication.

The sequence of DNA strand is written as 5 to 3 direction.

\*what is the nucleoside?

A nucleoside consists of a nitrogenous base covalently attached to a sugar (ribose or deoxyribose) but without the phosphate group. When nucleosides are phosphorylated by specific kinases (a type of enzyme in the cell), nucleotides are produced.



### Differences between DNA and RNA:

	<b>Stands For</b> DeoxyriboNucleic Acid.	RiboNucleic Acid.
<b>Definition</b>	A nucleic acid that contains the genetic instructions used in the development and functioning of all modern living organisms. DNA's genes are expressed, or manifested, through the proteins that its nucleotides produce with the help of RNA.	The information found in DNA determines which traits are to be created, activated, or deactivated, while the various forms of RNA do the work.

<b>Function</b>	The blueprint of biological guidelines that a living organism must follow to exist and remain functional. Medium of long-term, stable storage and transmission of genetic information.	Helps carry out DNA's blueprint guidelines. Transfers genetic code needed for the creation of proteins from the nucleus to the ribosome.
<b>Structure</b>	Double-stranded. It has two nucleotide strands which consist of its phosphate group, five-carbon sugar (the stable 2-deoxyribose), and four nitrogen-containing nucleobases: adenine, thymine, cytosine, and guanine.	Single-stranded. Like DNA, RNA is composed of its phosphate group, five-carbon sugar (the less stable ribose), and four nitrogen-containing nucleobases: adenine, uracil (not thymine), guanine, and cytosine.
<b>Location</b>	DNA is found in the nucleus of a cell (nuclear DNA) and in mitochondria (mitochondrial DNA).	Depending on the type of RNA, this molecule is found in a cell's nucleus (mRNA), its cytoplasm (tRNA), and its ribosome (rRNA).
<b>Stability</b>	Deoxyribose sugar in DNA is less reactive because of C-H bonds. Stable in alkaline conditions. DNA has smaller grooves, which makes it harder for enzymes to "attack."	Ribose sugar is more reactive because of C-OH (hydroxyl) bonds. Not stable in alkaline conditions. RNA has larger grooves, which makes it easier to be "attacked" by enzymes.
<b>Propagation</b>	DNA is self-replicating.	RNA is synthesized from DNA when needed.
<b>Unique Features</b>	The helix geometry of DNA is of B-Form. DNA is protected in the nucleus, as it is tightly packed.	The helix geometry of RNA is of A-Form. RNA strands are continually made, broken

DNA can be damaged by exposure to ultra-violet rays. RNA is down and reused. RNA is more resistant to damage by Ultra-violet rays.

**Genes:** is the basic physical and functional unit of heredity. Genes, which are made up of DNA, act as instructions to make molecules called proteins and RNA. Every person has two copies of each gene, one inherited from each parent. Most genes are the same in all people, but a small number of genes (less than 1 percent of the total) are slightly different between people. Alleles are forms of the same gene with small differences in their sequence of DNA bases. These small differences contribute to each person's unique physical features.

### **Genetic code:**

The nucleotide sequence of a gene's DNA specifies the amino acid sequence of a protein through the genetic code. Sets of three nucleotides, known as codons, each correspond to a specific amino acid. Additionally, a "start codon", and three "stop codons" indicate the beginning and end of the protein coding region. There are 64 possible codons (four possible nucleotides at each of three positions, hence  $4^3$  possible codons) and only 20 standard amino acids; hence the code is redundant and multiple codons can specify the same amino acid.

### **Mutation**

A mutation is a permanent change of the nucleotide sequence of the genome of an organism (chromosomal or gene structure). Mutations result from damage to DNA which is not repaired errors in the process of replication, or from the insertion or deletion of segments of DNA by mobile genetic elements. Mutations can be classified according to the causes to:

1-Spontaneous mutation: arises naturally and not as a result of exposure to mutagens. Also called natural mutation.

2-Induced mutation: produced by treatment with a physical or chemical agent that affects the DNA.

Mutations in chromosomal structure, including:

1-Amplifications (or gene duplications) leading to multiple copies of all chromosomal regions, increasing the dosage of the genes located within them.

2-Deletions of large chromosomal regions, leading to loss of the genes within those regions.

3-Chromosomal translocations: interchange of genetic parts from nonhomologous chromosomes.

4-Chromosomal inversions: reversing the orientation of a chromosomal segment.

Mutation affecting the gene structure, including:

1-Substitution mutations: exchange a single nucleotide for another.

2-Insertions: add one or more extra nucleotides into the DNA cause a shift in the reading frame (frameshift), which can significantly alter the gene product.

3-Deletions: remove one or more nucleotides from the DNA. Like insertions, these mutations can alter the reading frame of the gene.

## **Replication and transmission of DNA**

DNA replication follows a semi-conservative plan in which the original strand splits into two template strands from which two identical copies are built. DNA replication proceeds in three enzymatically catalyzed and coordinated steps: initiation, elongation and termination.

### **Initiation**

Replication of DNA starts with the ordered assembly of "initiator" proteins at special sites called Origin region of the Chromosome (OriC). Short stretches of DNA having a specific sequence of nucleotides at specific areas called a **replication forks**.

The replication fork is a Y-shaped structure that forms within the nucleus during DNA replication. It is created by helicases enzymes that untwist the double helix which break the hydrogen bonds holding the two DNA strands together. The resulting structure has two branches, each one made up of a single strand of DNA. These two strands serve as the template for the leading and lagging strands, Sequences used by these enzymes tend to be "AT-rich" (rich in adenine and thymine bases), because A-T base pairs have two hydrogen bonds (rather than the three formed in a C-G pair) which are easier to open. Once the origin has been located, these initiators recruit other proteins and form the pre-replication complex, which opens the double-stranded DNA.

### **Elongation**

DNA polymerase has 5'-3' activity and synthesis of new DNA by adding the free nucleotides in cytoplasm to a preexisting chain. All known DNA replication systems require a free 3' hydroxyl group before synthesis can be initiated.

The leading strand is the strand of nascent DNA which is being synthesized in the same direction as the growing replication fork.



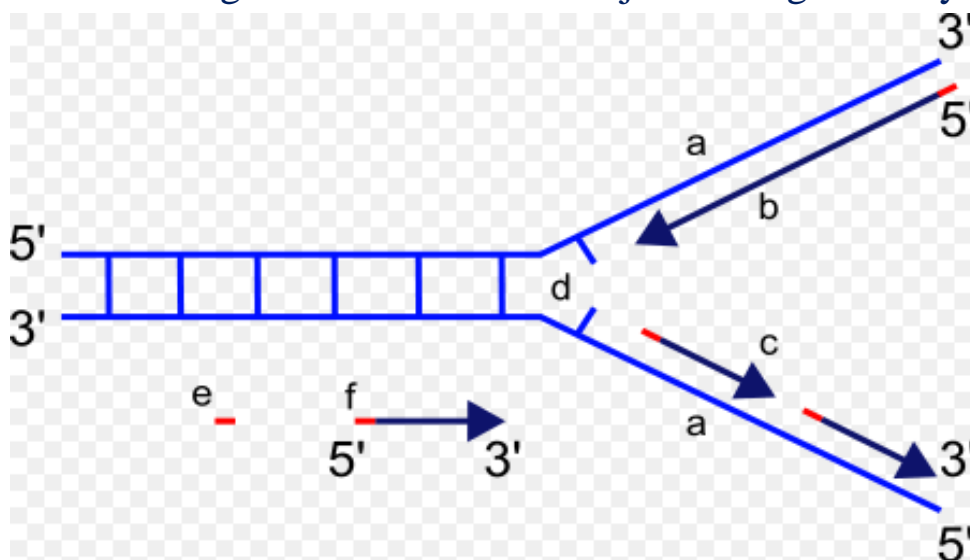
A polymerase "reads" the leading strand *template* and continuously adds complementary nucleotides to the nascent leading strand on a continuous basis.

\* In prokaryotes, the polymerase involved in leading strand synthesis is DNA polymerase III (DNA Pol III).

Primase provides a starting point for DNA polymerase to begin synthesis of the new DNA strand by adding RNA primers to the template strands.

The lagging strand is the strand of nascent DNA whose direction of synthesis is opposite to the direction of the growing replication fork. Because of its orientation, replication of the lagging strand is more complicated as compared to that of the leading strand.

The lagging strand is synthesized in short, separated segments. On the lagging strand *template*, a primase "reads" the template DNA and initiates synthesis of a short complementary RNA primer. A DNA polymerase extends the primed segments, forming Okazaki fragments. The RNA primers are then removed by RNase and replaced with DNA, and the fragments of DNA are joined together by DNA ligase.



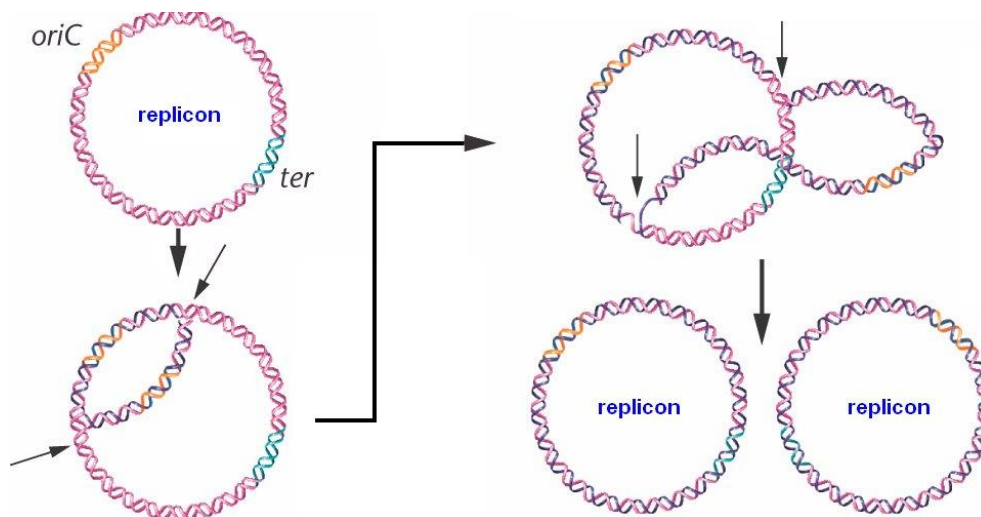
Scheme of the replication fork. a: template, b: leading strand, c: lagging strand, d: replication fork, e: primer, f: Okazaki fragments



## Termination

Eukaryotes initiate DNA replication at multiple points in the chromosome (linear chromosomes), so replication forks meet and terminate at many points in the chromosome.

Because bacteria have circular chromosomes, termination of replication occurs when the two replication forks meet each other on the opposite end of the parental chromosome. *E. coli* regulates this process through the use of termination sequences (*ter*). As a result, the replication forks are constrained to always meet within the termination region of the chromosome.



## Transmission of genetic material in prokaryotes:

### 1-Transformation (Uptake of DNA from environment)

Transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material (exogenous DNA) from its surroundings and taken up through the cell membrane (s). Transformation occurs naturally in some species of bacteria. For transformation to happen, bacteria must be in a state of

competence, which might occur as a time-limited response to environmental conditions such as starvation.

## **2. Transduction** (Virus mediated transfer of bacterial DNA)

Transduction is the process by which DNA is transferred from one bacterium to another by a virus. It also refers to the process whereby foreign DNA is introduced into another cell via a viral vector. Transduction does not require physical contact between the cell donating the DNA and the cell receiving the DNA (which occurs in conjugation), and it is DNase resistant (transformation is susceptible to DNase). Transduction is a common tool used by molecular biologists to stably introduce a foreign gene into a host cell's genome.

When bacteriophages (viruses that infect bacteria) infect a bacterial cell, the virus will take control of the cell's machinery for use in replicating its own viral DNA.

The new virus capsule now loaded with part bacterial DNA continues to infect another bacterial cell. This bacterial material may become recombined into another bacterium upon infection.

Medical Applications: Resistance to antibiotic drugs and correcting genetic diseases by direct modification of genetic errors.

## **3. Conjugation** (transfer of DNA between bacteria)

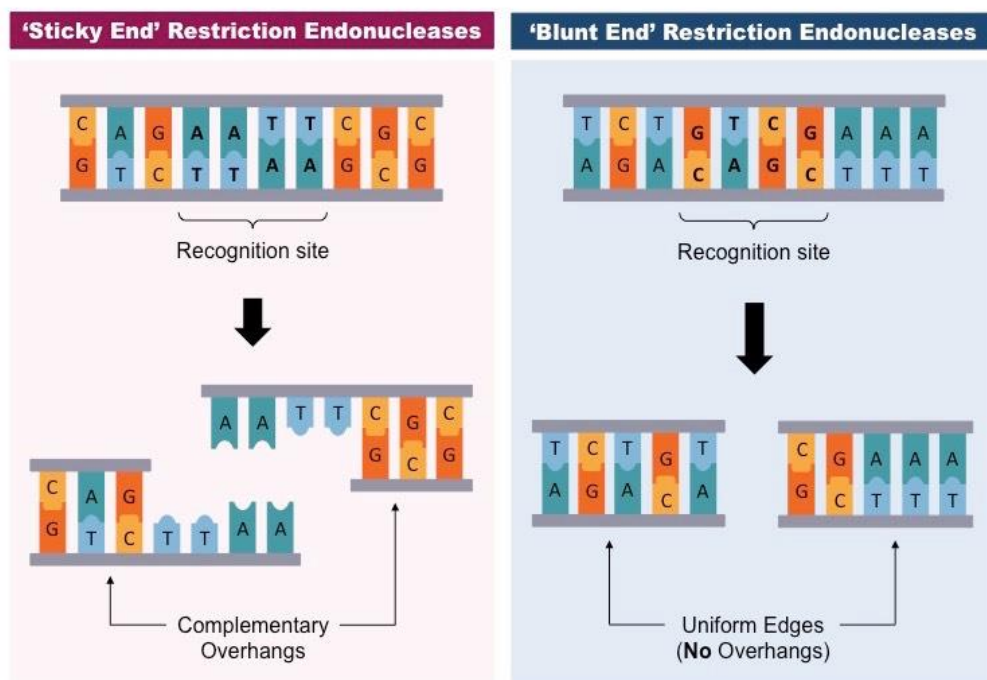
Conjugation is the process by which one bacterium transfers genetic material to another through direct contact. The donor bacterium carries a DNA sequence called the fertility factor, or F-factor. During conjugation, one bacterium serves as the donor (f+) of the genetic material, and the other serves as the recipient (f-). The F-factor allows the donor to produce a thin, tubelike structure called a pilus, which the donor uses to contact the recipient. The pilus then draws the two bacteria together, at which time the donor bacterium transfers genetic

material to the recipient bacterium. Typically, the genetic material is in the form of a plasmid, or a small, circular piece of DNA. The genetic material transferred during conjugation often provides the recipient bacterium with some sort of genetic advantage. For instance, in many cases, conjugation serves to transfer plasmids that carry antibiotic resistance genes.

## Genetic engineering

Many bacteria produce restriction endonucleases (restriction enzymes RE). These enzymes have become very important tools in genetic engineering especially in cloning when inserts DNA into vector DNA.

These enzymes often cut the double stranded DNA at specific sequences called Pallindrome sequence to generate sticky or blunt ends which allow molecular geneticists to connect DNA fragments together from different sources. Example: *EcoRI* enzyme isolated from species of *E. coli* generate sticky ends, while *EcoRV* enzyme give blunt ends.



The main steps of a cloning protocol:

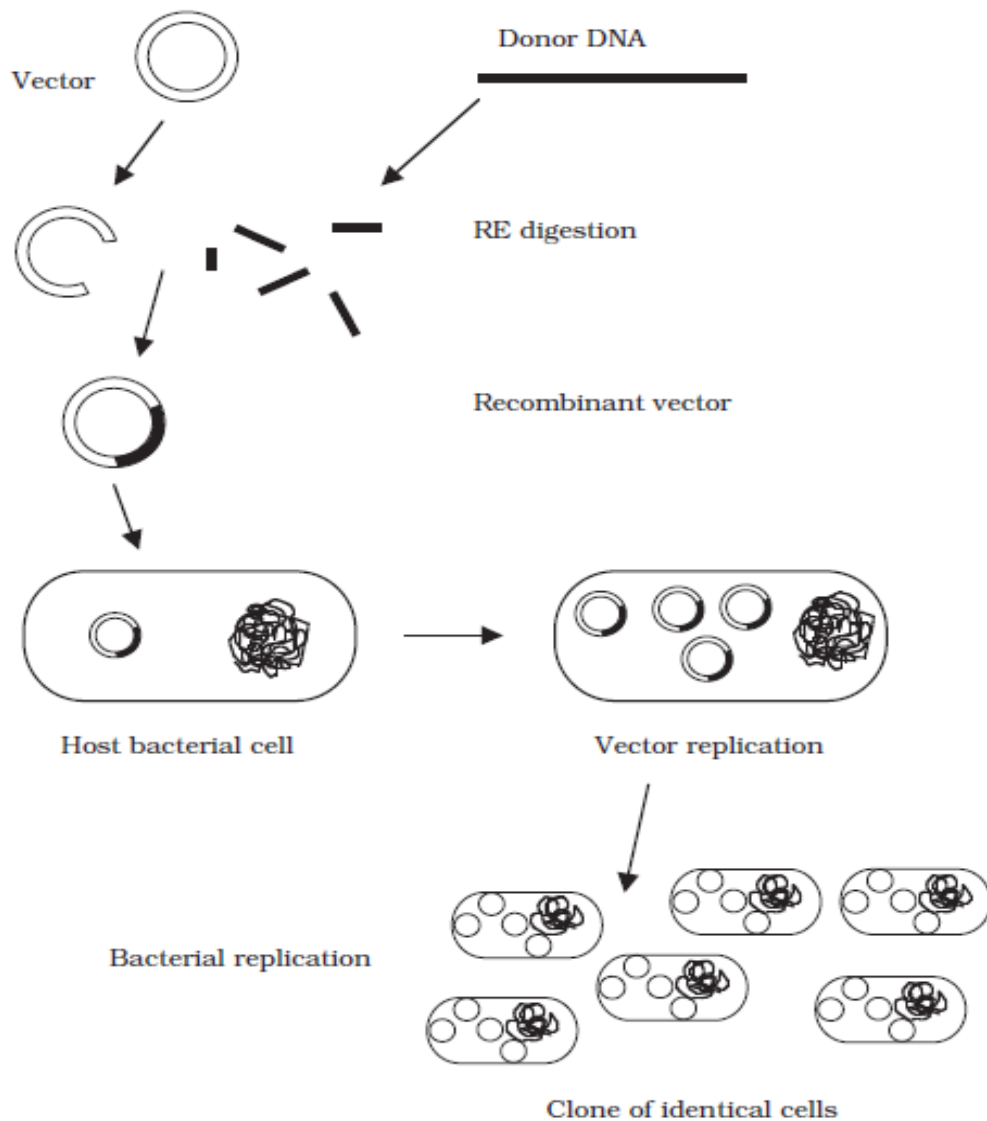
'donor' DNA and vector are digested with RE to provide compatible sticky ends.

a fragment of donor DNA is spliced into the vector molecule.

the recombinant vector gains entry to a host cell (e.g. *E. coli* ).

the vector replicates inside the cell, making further copies of the inserted DNA.

host multiplication results in the formation of a clone of cells, all containing the same recombinant plasmid.



*P. aeruginosa* is a common Gram-negative aerobic, bacillus bacterium with unipolar motility that can cause disease in plants and animals, including humans (opportunistic). It is found in soil, water, skin flora, and most man-made environments throughout the world.

*P. aeruginosa* is citrate, catalase, and oxidase positive. It can secrete a variety of pigments, including pyocyanin (blue-green), pyoverdine (yellow-green and fluorescent), and pyorubin (red-brown).

Definitive clinical identification of *P. aeruginosa* often includes identifying the production of both pyocyanin and fluorescein, as well as its ability to grow at 42°C. *P. aeruginosa* is capable of growth in diesel and jet fuels, where it is known as a hydrocarbon-using microorganism. The genome of *P. aeruginosa* is relatively large (6–7 Mb).

Cell-surface polysaccharides play diverse roles in the bacterial "lifestyle". They serve as a barrier between the cell wall and the environment, mediate host-pathogen interactions, and form structural components of biofilms.

### **Pathogenesis**

An opportunistic, nosocomial pathogen of immunocompromised individuals, *P. aeruginosa* typically causes the respiratory infections, urinary tract infections, wound infections, endocarditis, gastrointestinal infections and also causes other blood infections. It is the most common cause of infections of burn injuries and of the outer ear (otitis externa), and is the most frequent colonizer of medical devices (e.g., catheters). *Pseudomonas* can be spread by equipment that gets contaminated and is not properly cleaned or on the hands of healthcare workers. Lipopolysaccharide plays a direct role in causing fever, shock, oliguria and leukopenia.

### **Virulence factors**

*P. aeruginosa* secretes many virulent factors to colonize the cells of its host.

### Adhesion:

- Polysaccharides capsule (glycocalyx)
- Alginate slime (biofilm) forms a viscous gel around the bacteria, may act as an adhesion and prevent the phagocytosis
- flagella and pili (motility/chemotaxis) play a vital role in the infection of burns and wounds).

### Invasion:

- elastase, an extracellular zinc protease, attacks eukaryotic proteins such as collagen and elastin and destroys the structural proteins of the cell, also breaks down human immunoglobulin and serum alpha proteins.
- Alkaline protease
- Hemolysins (phospholipase and lecithinase)
- cytotoxin (leukocidin)
- siderophores (Iron capturing ability)
- pyocyanin diffusible pigment

### Toxins:

- exotoxin A, the most toxic protein produced by *P. aeruginosa*, which inhibits the protein synthesis of the host's cells.
- Lipopolysaccharides as endotoxin.
- Exoenzyme S: interferes with phagocytic killing.

Antimicrobial resistance: due to outer membrane changes.

### **Diagnosis:**

Specimens from skin lesions, pus, urine, blood, spinal fluid, sputum, and other material should be obtained as indicated by the type of infection.

- Gram stain
- Inability to ferment lactose
- positive oxidase reaction

- Fruity odor
- Ability to grow at 42c.
- Fluorescence under UV radiation

## **Treatment**

*P. aeruginosa* is frequently isolated from nonsterile sites (mouth swabs, sputum, etc.).

Susceptibility testing is essential, combination of gentamicin and carbenicillin can be very effective in patients with acute *Pseudomonas* infections.

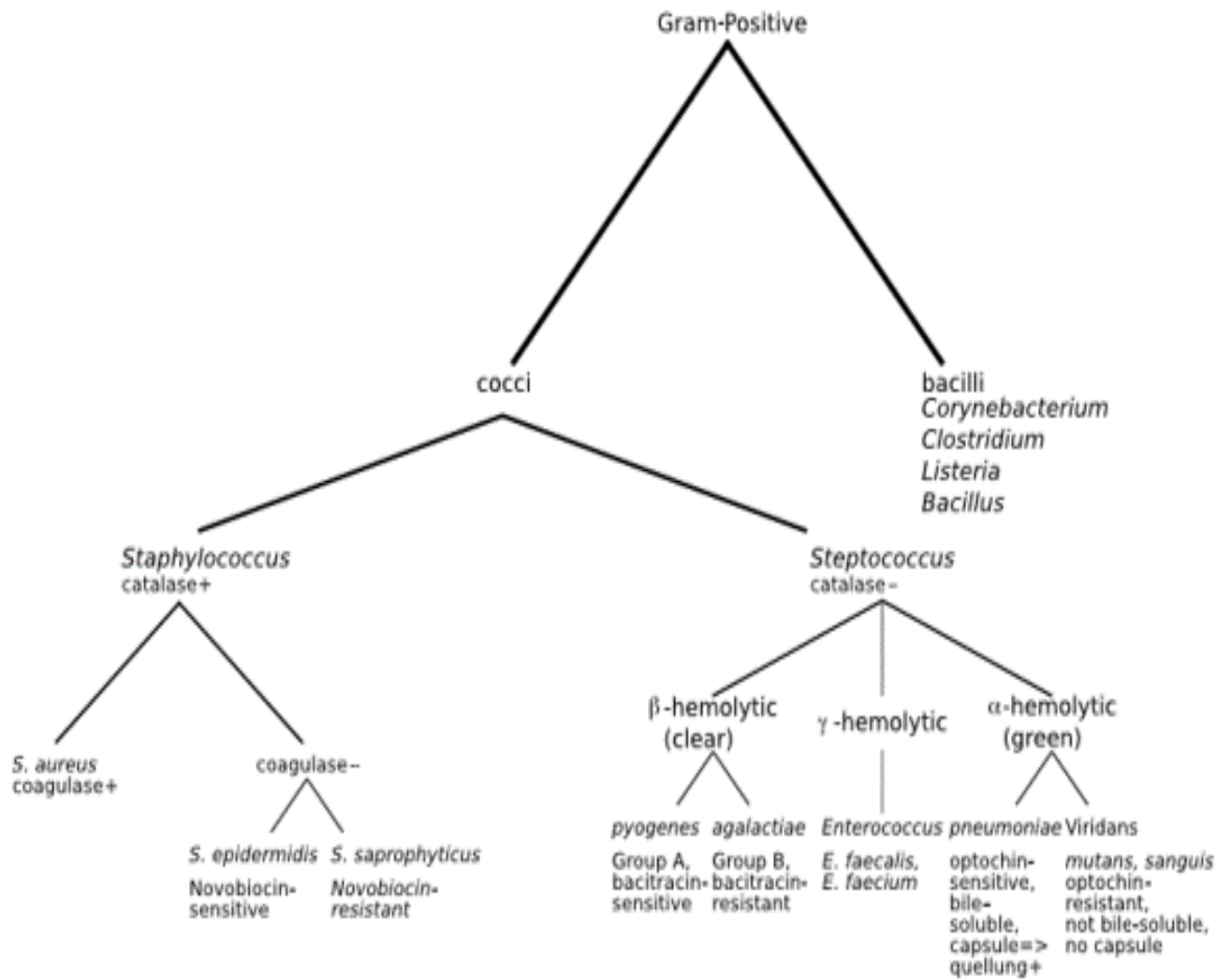
Development of multidrug resistance by *P. aeruginosa* isolates requires several different genetic events, including acquisition of different mutations and/or horizontal transfer of antibiotic resistance genes. Therefore, research for the discovery of new antibiotics and drugs against *P. aeruginosa* is very much needed. Phage therapy against *P. aeruginosa* has been investigated as a possible effective treatment, which can be combined with antibiotics, has no contraindications and minimal adverse effects. Phages are produced as sterile liquid, suitable for intake, applications etc.

In burn patients, topical therapy with antimicrobial agents as silver sulfadiazine coupled with surgical debridement, has markedly reduced sepsis.

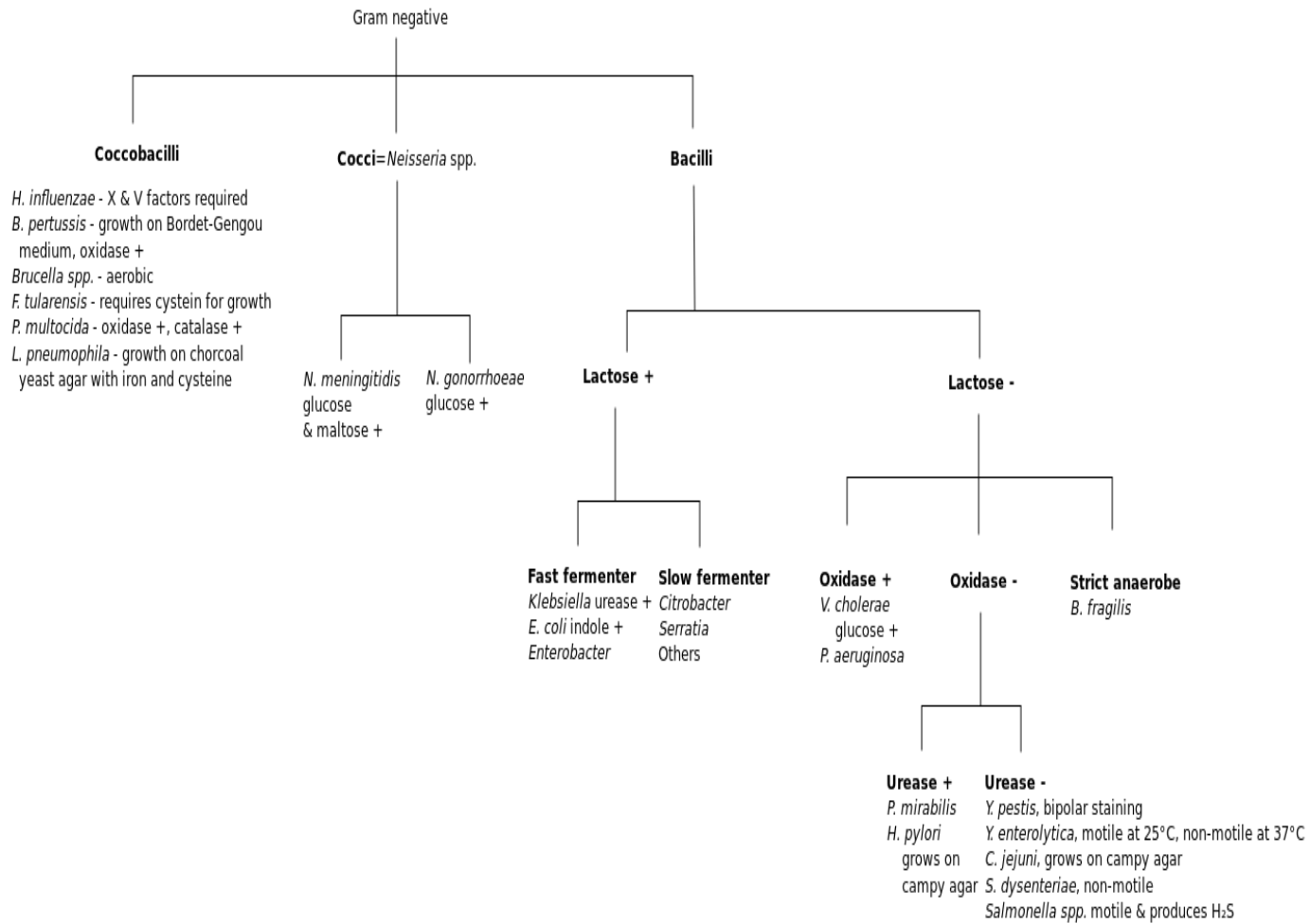
## **Prevention**

Avoiding hot tubs because *P. aeruginosa* can survive in hot temperatures. Avoiding pools that may be poorly maintained and keep contact lens equipment and solutions from becoming contaminated. Washing your hands often can benefit as well with contact to many other pathogen infections.









## Lecture 5 (Ch6) - Viruses

- Topics
  - Characteristics
  - Structure/Classification
  - Multiplication
  - Cultivation and replication
  - Nonviral infectious agents
  - Treatment



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## Virus Characteristics

- obligate intracellular parasites
- not cells
- tiny! - 20nm -450nm (no light scope)
- do not independently fulfill characteristics of life
- active only inside the cell
- surface molecules confer high specificity
- use hosts genetic material
- lack enzymes or machinery for synthesis

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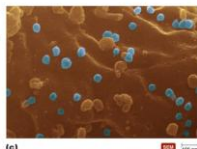
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## Viral Host Range



Most infect only specific host (attachment)  
Can be so specific only infect specific type of cell in specific host  
Some generalists – infect many kinds of cells in many different hosts

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## Structure

- Size and morphology
- Capsid
- Envelope
- Complex
- Nucleic acid

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## Virus- inside & out

- **Extracellular**
  - Called virion
  - Protein coat (capsid) surrounding nucleic acid
  - Nucleic acid and capsid also called nucleocapsid
  - Some have phospholipid envelope
  - Outermost layer provides protection and recognition sites for host cells
- **Intracellular**
  - Capsid removed
  - Virus exists as nucleic acid

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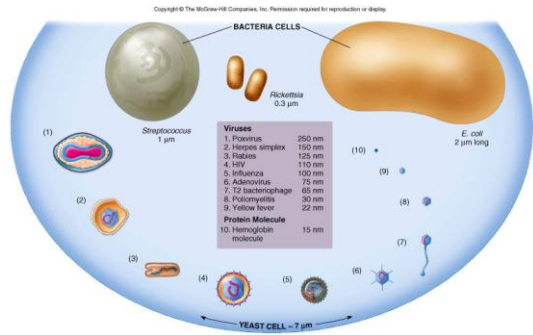
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## Size Comparison



Size comparison of viruses

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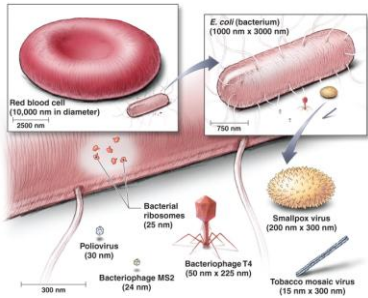
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## More Size...



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## Looking at Virus

Electron microscopy, "negative" staining, positive staining, and shadow casting are methods of viewing viruses.

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a)

b)

c)

E.M. methods of viewing viruses

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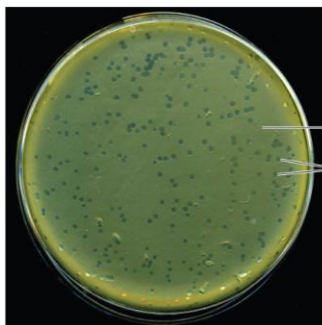
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## Visualizing Virus Indirectly



Bacterial lawn

Viral plaques

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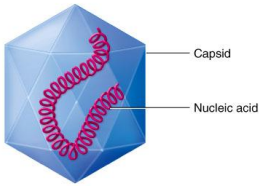
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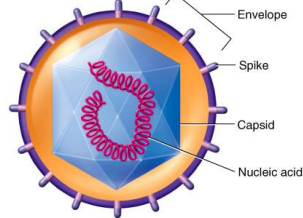
The two major structure types for viruses:

naked nucleocapsid virus



(a) Naked Nucleocapsid Virus

enveloped virus



(b) Enveloped Virus

Generalized viral structures

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## Capsid

- Protective outer shell that surrounds viral nucleic acid
- Capsid spikes
- Composed of capsomer subunits
- Two types of capsids (based on shape):
  - Helical
  - Icosahedral

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## Helical capsid

- Naked helical virus
  - Nucleocapsid is rigid and tightly wound into a cylinder-shaped package
  - Example: Tobacco mosaic virus
- Enveloped helical virus
  - Nucleocapsid is more flexible than naked virus
  - Examples: Influenza, measles, rabies

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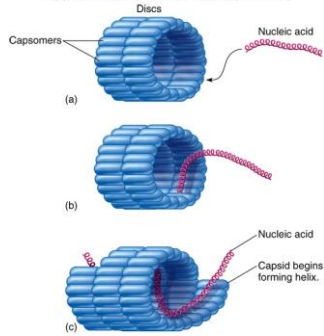
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**Helical capsids:**

rod-shaped capsomers form hollow discs, like a bracelet.

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Helical nucleocapsid assembly

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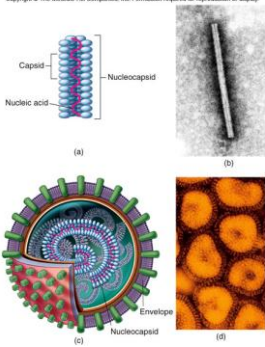
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**Comparison: naked helical plant virus and an enveloped helical human virus.**

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Typical variation of viruses with helical Nucleocapsids.

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**Icosahedron capsid**

- Three-dimensional, 20-sided with 12 evenly spaced corners
- Variation in capsomer number
  - Polio virus 32 capsomers
  - Adenovirus 240 capsomers

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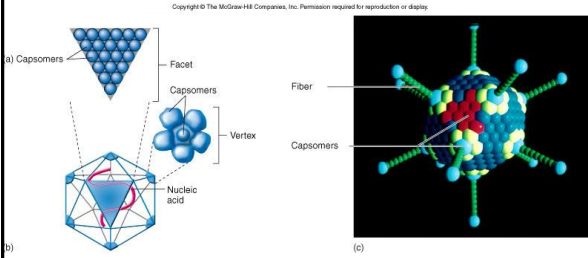
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## The structure and formation of an adenovirus



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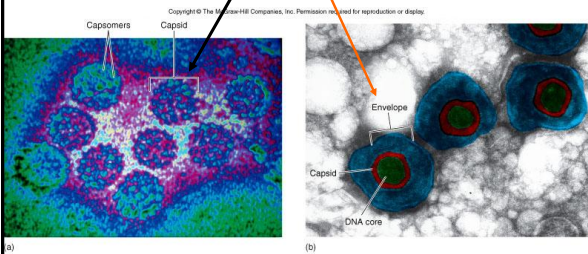
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## Icosahedral viruses – can be naked or enveloped.



Two types of icosahedral viruses

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## Viral Envelope

- Lipid and proteins
- Envelope spikes
- During release of animal viruses, a part of the host membrane is taken
- Enable pleomorphic shape of the virus
  - Spherical
  - Filamentous
- Recognition & Attachment

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## Function of the capsid/envelope

- Protect nucleic acid from the host's acid- and protein-digesting enzymes
- Assist in binding and penetrating host cell
- Stimulate the host's immune system

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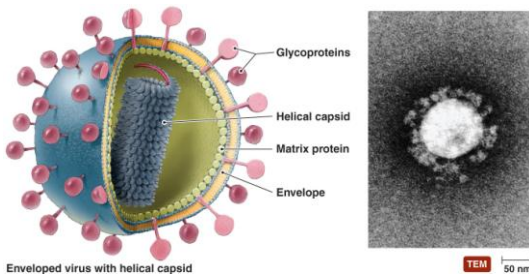
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## An Enveloped Virus



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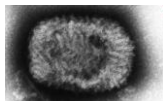
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## Complex viruses

- Structure is more intricate than helical or icosahedral viruses.

Examples:

- Pox virus
  - Several layers of lipoproteins
  - Course surface fibrils
- Bacteriophage (next slide)
  - Polyhedral head
  - Helical tail
  - Fibers for attachment



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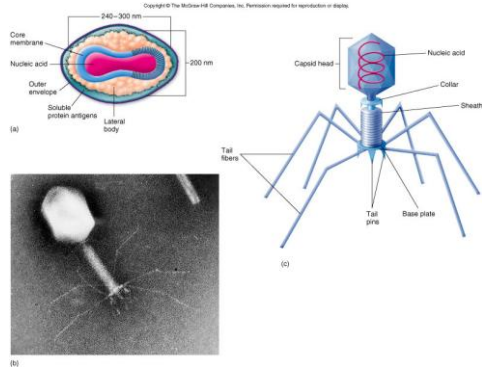
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### Pox virus and T4 bacteriophage



Detailed structure of complex viruses

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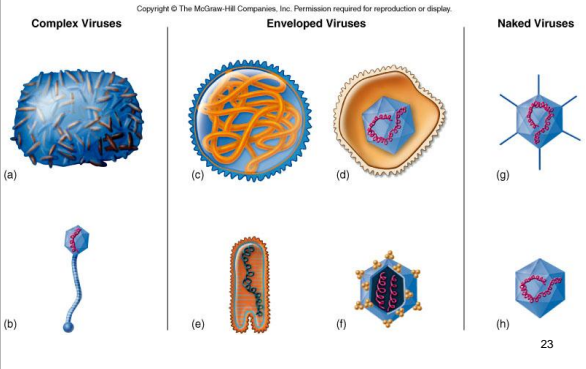
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### Morphology: (helical, icosahedral, complex) of a naked virus, enveloped virus and a complex virus.




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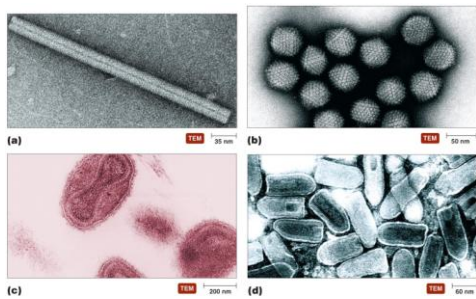
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### Virion Shapes in EM




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## Viral nucleic acid

- Viruses contain either DNA or RNA
- Possess only the genes to invade and regulate the metabolic activity of host cells
  - Examples:
    - Hepatitis B (DNA)(4 genes)
    - Herpesviruses (DNA) (100 genes)
    - Rotavirus (dsRNA)
    - Coronavirus, SARs (ssRNA)
- No viral metabolic genes, because uses host's metabolic resources

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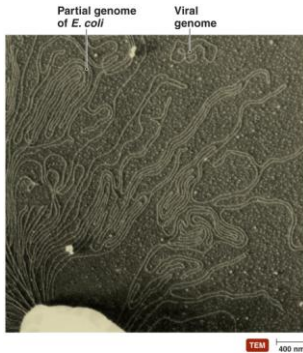
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## Compare Genome Size



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## The 7 classes of virus

- DNA viruses contain classes I, II, and VII
- RNA viruses contain classes III-VI.

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## DNA virus classes (I, II, VII)

- Class I viruses:
  - double-stranded DNA (dsDNA) genome
  - Examples:
    - Some phages
    - Family *Herpesviridae* (includes human herpesviruses), *Varicella Zoster*, *Poxviridae*, JC, *papilloma*
- Class II viruses:
  - +sense single-stranded DNA (ssDNA) genome.
  - Example: *Parvoviridae*
- Class VII viruses:
  - double-stranded, reverse transcriptase (dsDNA-RT) genome.
  - Example: *Hepadenovirus*

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## RNA virus classes (III-VI)

- Class III viruses:
  - double-stranded RNA (dsRNA) genome.
  - Example: *Reovirus*
- Class IV viruses:
  - +sense single-stranded RNA (ssRNA) genome (acts as mRNA).
  - Example: *Picornaviruses*,
- Class V viruses:
  - -sense single-stranded RNA (ssRNA) genome used as a template for mRNA synthesis.
  - Example: *Rhabdovirus*
- Class VI viruses:
  - +sense single- stranded reverse transcriptase RNA (ssRNA-RT) genome (with DNA intermediate in replication and also mRNA synthesis).
  - Example: *Retroviridae*

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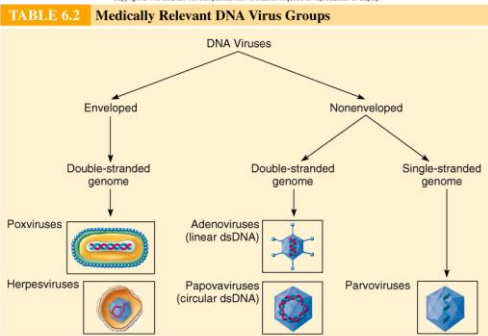
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### Examples of medically important DNA viruses



Adapted from: *Poxviridae* from Buller et al., National Institute of Allergy & Infectious Disease, Department of Health & Human Services. 30

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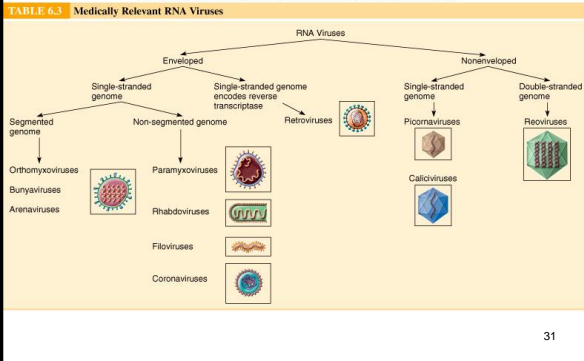
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## Some medically important RNA viruses

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## Virus & Cancer

- Animal's genes dictate some cells can no longer divide and those that can divide are prevented from unlimited division
- Genes for cell division "turned off" or genes inhibiting division "turned on"
- Neoplasia
  - Uncontrolled cell division in multicellular animal; mass of neoplastic cells is tumor
- Benign vs. malignant tumors
  - Metastasis
  - Cancers

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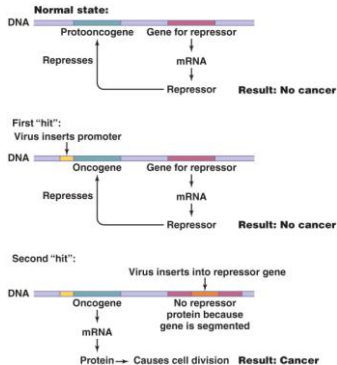
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## Oncogene Theory

(induction of cancer in humans)




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## Environmental Factors, Viruses & Cancer

- Environmental factors that contribute to the activation of oncogenes
  - Ultraviolet light
  - Radiation
  - Carcinogens
  - Viruses

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## Virus & Cancer

- Viruses cause **20–25%** of human cancers
  - Some carry copies of oncogenes as part of their genomes
  - Some promote oncogenes already present in host
  - Some interfere with tumor repression when inserted into host's repressor gene
- Specific viruses are known to cause ~15% of human cancers
  - Burkitt's lymphoma
  - Hodgkin's disease
  - Kaposi's sarcoma
  - Cervical cancer

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## Classification

- Structure
- Chemical composition
- Genetic makeup
- Host relationship
- Type of disease

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## Three orders of viruses developed for classification.

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**TABLE 6-4** Examples from the Three Orders of Viruses

Genome Type	Order	Family	Genus	Species
dsDNA	Caudovirales	Poxviridae	Orthopoxvirus	Vaccinia virus
neg ssRNA	Mononegavirales	Paramyxoviridae	Morbillivirus	Measles virus
pos (ss)RNA	Nidovirales	Togaviridae	Rubivirus	Rubella virus

Adapted from van Regenmortel, M., ed. 2000. *Virus Taxonomy, Seventh Report of the International Committee on Taxonomy of Viruses*. New York: Academic Press.

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## Classification of important human viruses

**TABLE 6-5** Important Human Virus Families, Genera, Common Names, and Types of Diseases

Family	Genus of Virus	Common Name of Genus Members	Name of Disease
<b>DNA Viruses</b>	Poxviridae	Orthopoxvirus	Smallpox, cowpox
		Myxoma (Myx) 1 virus	Myxomatosis, rabbit fever
		Myxoma (Myx) 2 virus	Congo fever
		Myxoma (Myx) 3 virus	Chikungunya fever
		Myxoma (Myx) 4 virus	AIDS
		Myxoma (Myx) 5 virus	Adenovirus infection
		Myxoma (Myx) 6 virus	Herpesvirus infection
		Myxoma (Myx) 7 virus	Herpesvirus infection
		Myxoma (Myx) 8 virus	Herpesvirus infection
		Myxoma (Myx) 9 virus	Herpesvirus infection
<b>DNA Viruses</b>	Herpesviridae	Herpesvirus	Herpes
		Herpesvirus	Herpes
		Herpesvirus	Herpes
		Herpesvirus	Herpes
		Herpesvirus	Herpes
		Herpesvirus	Herpes
		Herpesvirus	Herpes
		Herpesvirus	Herpes
		Herpesvirus	Herpes
		Herpesvirus	Herpes
<b>RNA Viruses</b>	Flaviviridae	Flavivirus	Yellow fever
		Flavivirus	Yellow fever
		Flavivirus	Yellow fever
		Flavivirus	Yellow fever
		Flavivirus	Yellow fever
		Flavivirus	Yellow fever
		Flavivirus	Yellow fever
		Flavivirus	Yellow fever
		Flavivirus	Yellow fever
		Flavivirus	Yellow fever

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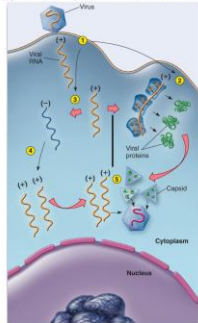
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## Replication/Multiplication

- Adsorption
- Penetration
- Uncoating
- Synthesis
- Assembly
- Release

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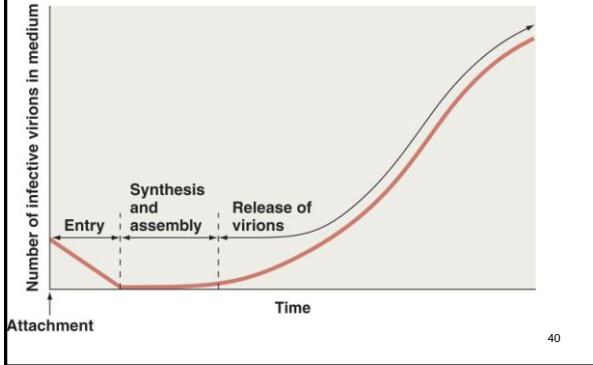
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## Virions in Persistent Infection




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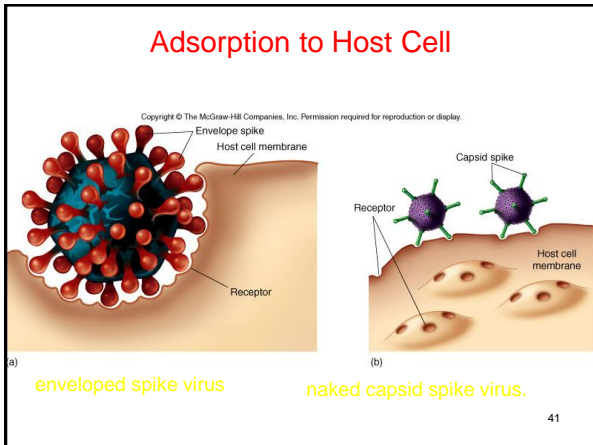
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## Adsorption to Host Cell




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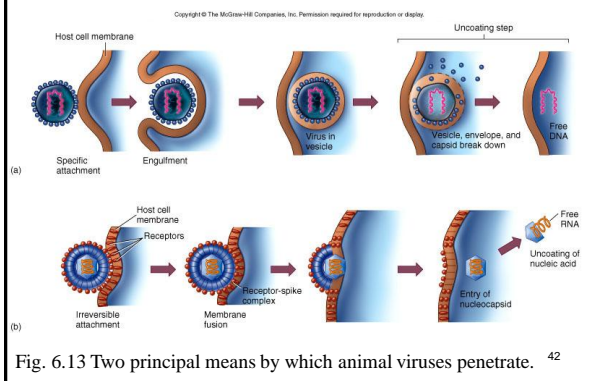
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## Penetration of animal viruses occur by endocytosis or fusion between the viral envelope and the the host cell membrane




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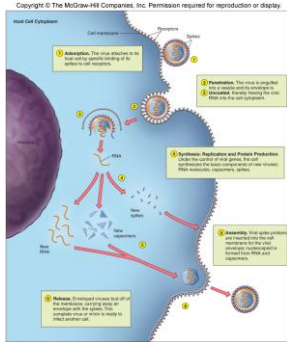
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**Uncoating and synthesis of viruses rely on the host's metabolic systems.**



Multiplication cycle general features of enveloped animal virus.

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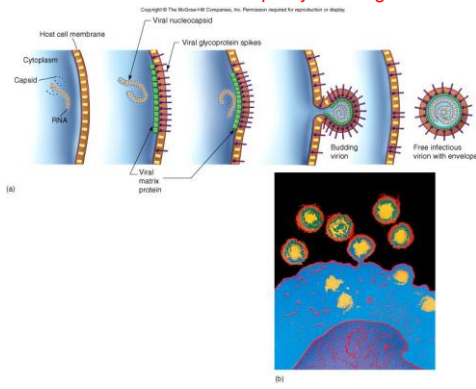
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**A mature virus can obtain an envelope by budding off the host cell.**



Maturation and release of enveloped viruses

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**Cytopathic effects**

- Damage to the host cell due to a viral infection
  - Inclusion bodies
  - Syncytia
  - Chronic latent state
  - Transformation

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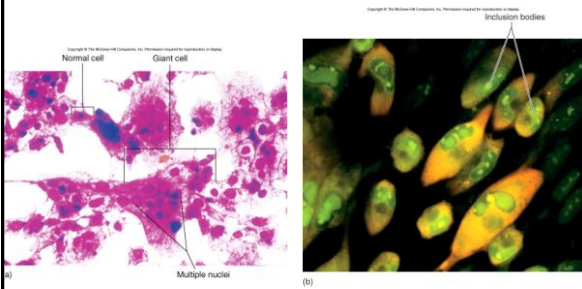
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## Syncytia and inclusion bodies



Cytopathic changes in cells and cell cultures infected by viruses 46

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## Bacteriophage

- Bacterial virus
- Multiplication is similar to animal viruses except for the penetration (inject DNA), release (lyses) and prophage (lysogeny) stages
- Useful as alternate therapy

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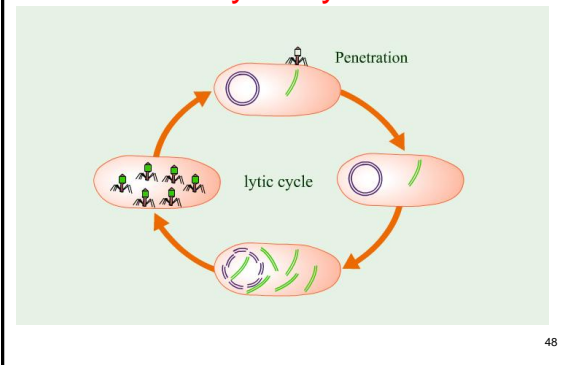
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## Lytic Cycle



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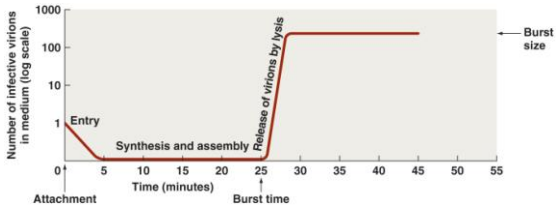
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## Viral Lytic Cycle



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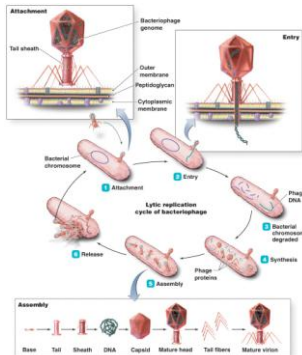
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## Viral Lytic Cycle



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## Lysogeny

- Modified replication cycle
- Infected host cells grow and reproduce normally for generations before they lyse
- Temperate phages
  - Prophages – inactive phages
- Lysogenic conversion results when phages carry genes that alter phenotype of a bacterium

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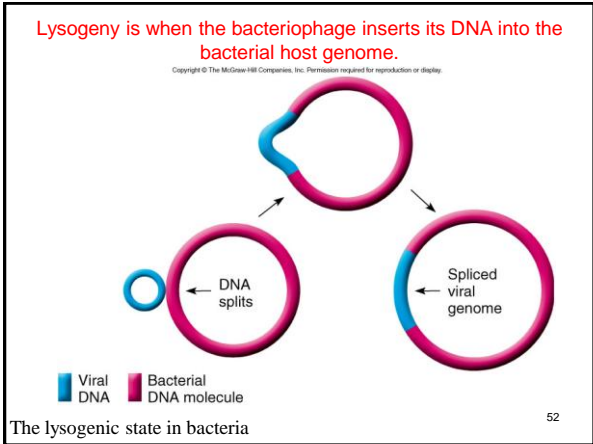
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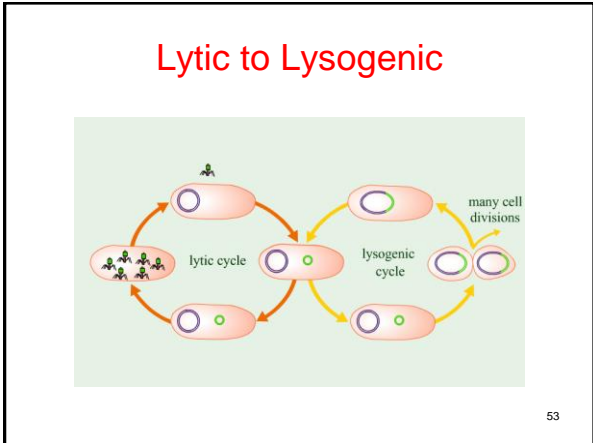
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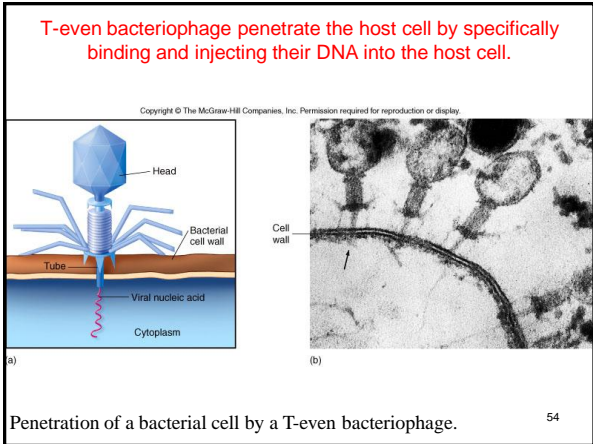
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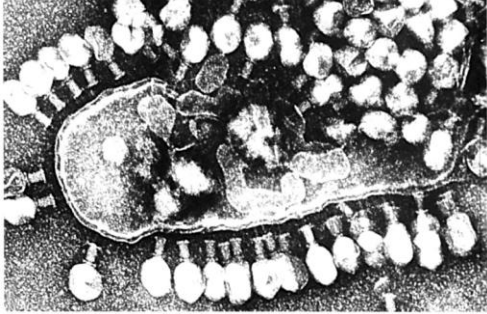
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After viral multiplication inside the host cell, viral enzymes weaken the host cell membrane, lyse the cell, and release virions

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A weakened bacterial cell, crowded with viruses.

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Comparison: bacteriophage and animal virus multiplication

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TABLE 6.7 Comparison of Bacteriophage and Animal Virus Multiplication

	Bacteriophage	Animal Virus
<b>Adsorption</b>	Precise attachment of special tail fibers to cell wall	Attachment of capsid or envelope to cell surface receptors
<b>Penetration</b>	Injection of nucleic acid through cell wall; no uncoating of nucleic acid	Whole virus is engulfed and uncoated, or virus surface fuses with cell membrane, nucleic acid is released
<b>Synthesis and Assembly</b>	Occurs in cytoplasm Cessation of host synthesis  Viral DNA or RNA is replicated and begins to function Viral components synthesized	Occurs in cytoplasm and nucleus Cessation of host synthesis Viral DNA or RNA is replicated and begins to function Viral components synthesized
<b>Viral Persistence</b>	Lysogeny	Latency, chronic infection, cancer
<b>Release from Host Cell</b>	Cell lyses when viral enzymes weaken it	Some cells lyse; enveloped viruses bud off host cell membrane
<b>Cell Destruction</b>	Immediate	Immediate or delayed

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Cultivation and Replication

- *In vivo* methods
  - Laboratory animals
  - Embryonic bird tissues
- *In vitro* methods
  - Cell or tissue culture

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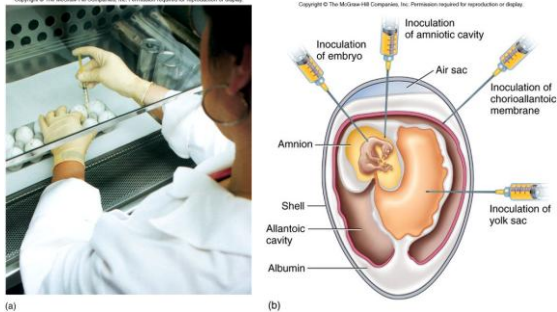
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Early developing bird embryos have a protective case that provides an ideal viral propagation environment



Cultivating animal viruses in a developing bird embryo

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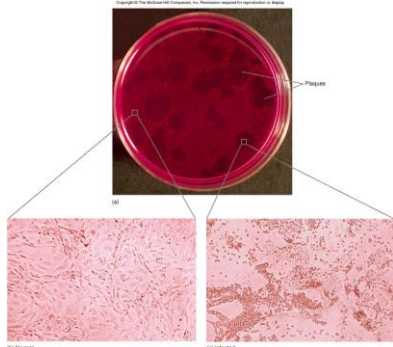
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A monolayer of monkey kidney cells is a cell culture that enables virus propagation



Normal and infected cell cultures

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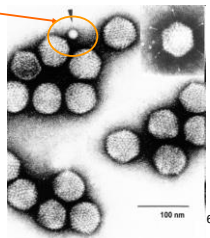
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Noncellular Infectious Agents

- **Prions** (naked proteins)
- **Satellite viruses** (usually plant virus associated for purpose of replication)
- **Viroids** (unique plant pathogens, small, single-stranded, circular RNA )



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## Prions

- Protein particle with no nucleic acid, no envelope, no capsid
- Diseases
  - Creutzfeldt-Jakob
  - “mad cow disease”



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## Prion Diseases

- Fatal neurological degeneration, fibril deposits in brain, and loss of brain matter
- Large vacuoles form in brain
  - Characteristic spongy appearance
- Spongiform encephalopathies – BSE, vCJD, kuru
- Prions only destroyed by incineration or autoclaving in 1 N NaOH

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## Prions (cont...)

- Cellular PrP protein
  - Made by all mammals
  - Normal structure with  $\alpha$ -helices called cellular PrP
- Prion PrP
  - Disease-causing form with  $\beta$ -sheets called prion PrP
- Prion PrP converts cellular PrP into prion PrP by inducing conformational change

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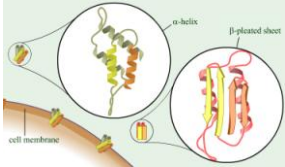
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## Prion Protein Folding

- Normally, nearby proteins and polysaccharides force PrP into cellular shape
- Excess PrP or PrP mutations result in formation of prion PrP
  - Cause newly synthesized cellular PrP to refold into prion PrP



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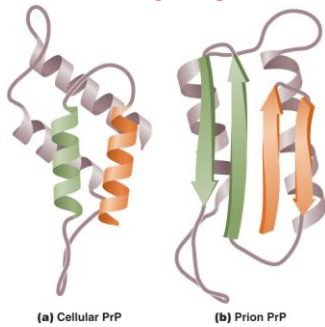
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## Stable Prion Protein (PrP) Forms



(a) Cellular PrP

(b) Prion PrP

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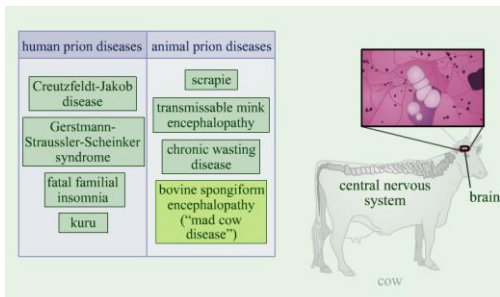
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## The Prion Diseases



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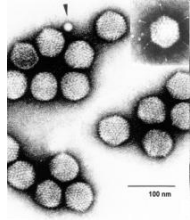
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## Satellite viruses

- Dependent on other viruses for replication
- Ex. Delta agent, which is only expressed in the presence of hepatitis B virus, depend on it for replication- the only viroid like infectious agent of animals.



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## Viroids

- Plant pathogens
  - Tomatoes, potatoes, cucumbers.
- 1/10<sup>th</sup> the size of normal viruses
- Naked strands of RNA, no capsid

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## Example Viroid Effects



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The staphylococci are gram-positive spherical cells, usually arranged in grapelike irregular clusters. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. The staphylococci produce catalase, which differentiates them from the streptococci. Pathogenic staphylococci produce many extracellular substances.

Staphylococci are relatively resistant to drying, heat (they withstand 50°C for 30 minutes), and 9% sodium chloride but are readily inhibited by certain chemicals, e. g, 3% hexachlorophene. Some are members of the normal flora of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia. Staphylococci rapidly develop resistance to many antimicrobial agents.

### **Antigenic Structure**

Staphylococci contain antigenic polysaccharides and proteins as well as other substances important in cell wall structure.

1-Peptidoglycan, is important in the pathogenesis of infection: It elicits production of interleukin-1 (endogenous pyrogen) and opsonic antibodies by monocytes, and it can be a chemoattractant for polymorphonuclear leukocytes, and activate complement.

2- Teichoic acids, are linked to the peptidoglycan and can be antigenic. Antiteichoic acid antibodies may be found in patients with active endocarditis due to *S aureus*.

3-Protein A is a cell wall component, important virulence factors. Protein A binds to the Fc portion of IgG molecules.

4-Some *S aureus* strains have capsules, which inhibit phagocytosis by polymorphonuclear leukocytes unless specific antibodies are present. Most strains of *S aureus* have coagulase, or clumping factor, on the cell wall surface.

The genus *Staphylococcus* has at least 40 species. The three most frequently encountered species of clinical importance are *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*.

### ***Staphylococcus aureus***

*S aureus* is **coagulase-positive**, which differentiates it from the other species. *S aureus* is a major pathogen for humans. Almost every person will have some type of *S aureus* infection during a lifetime, ranging in severity from food poisoning or minor skin infections to severe life-threatening infections.

### **Enzymes & Toxins**

Staphylococci can produce disease both through their ability to multiply and spread widely in tissues and through their production of many extracellular substances.

#### **-Catalase**

Converts hydrogen peroxide into water and oxygen. The catalase test differentiates the staphylococci, which are positive, from the streptococci, which are negative.

#### **-Coagulase & Clumping Factor**

An enzyme-like protein that clots plasma. Coagulase binds to prothrombin; together they become enzymatically active and initiate fibrin polymerization. Coagulase production is considered synonymous with invasive pathogenic potential. Clumping factor is responsible for

adherence of the organisms to fibrinogen and fibrin. When mixed with plasma, *S aureus* forms clumps. Clumping factor is distinct from coagulase.

### **-Other Enzymes**

Include a hyaluronidase, or spreading factor; a staphylokinase; proteinases; lipases; and  $\beta$ -lactamase.

### **-Exotoxins**

The  $\alpha$ -toxin is a potent hemolysin that acts on a broad spectrum of eukaryotic cell membranes. The  $\beta$ -toxin is toxic for many kinds of cells, including human RBCs. The  $\gamma$ -toxin (Panton-Valentine Leukocidin) has two components designated as S and F. It can kill white blood cells of humans and rabbits by causing pore formation in the cellular membranes that increase cation permeability. This leads to massive release of inflammatory mediators which are responsible for necrosis and severe inflammation. This toxin is an important virulence factor in community associated methicillin resistant *S aureus* (MRSA) infections. The  $\delta$ -toxin disrupts biologic membranes and may have a role in *S aureus* diarrheal diseases.

### **Exfoliative Toxins**

These epidermolytic toxins of *S aureus* yield the generalized desquamation of the staphylococcal scalded skin syndrome by dissolving the mucopolysaccharide matrix of the epidermis. The toxins are superantigens.

### **Toxic Shock Syndrome Toxin**

Most *S aureus* strains isolated from patients with toxic shock syndrome produce a toxin called **toxic shock syndrome toxin-1** (TSST-1), which is the prototypical **superantigen**. The toxin is associated with fever, shock, and multisystem involvement, including a desquamative skin rash. The gene for TSST-1 is found in about 20% of *S aureus* isolates, including MRSA.

## **Enterotoxins**

There are multiple (A–E, G–J, K–R and U, V) enterotoxins. Approximately 50% of *S aureus* strains can produce one or more of them. Like TSST-1, the enterotoxins are superantigens. The enterotoxins are heat-stable and resistant to the action of gut enzymes. Important causes of food poisoning, enterotoxins are produced when *S aureus* grows in carbohydrate and protein foods. Ingestion of 25 µg of enterotoxin B results in vomiting and diarrhea.

The exfoliative toxins, TSST-1, and the enterotoxin genes are on a chromosomal element called a pathogenicity island. It interacts with accessory genetic elements—bacteriophages—to produce the toxins.

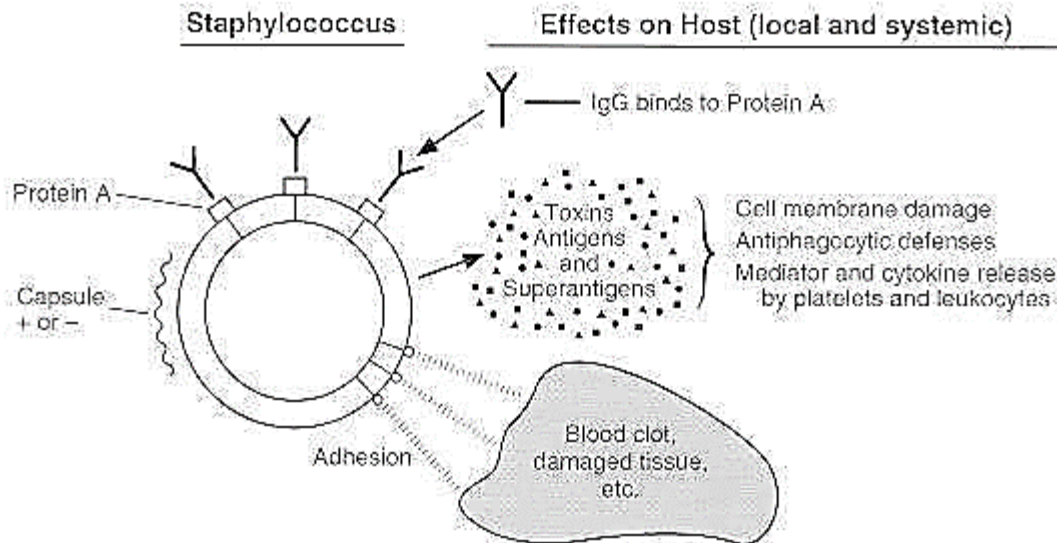
## **Pathogenesis**

Staphylococci, particularly *S epidermidis*, are members of the normal flora of the human skin and respiratory and gastrointestinal tracts. Nasal carriage of *S aureus* occurs in 20–50% of humans. Staphylococci are also found regularly on clothing and fomites in human environments.

The pathogenic capacity of a given strain of *S aureus* is the combined effect of extracellular factors and toxins together with the invasive properties of the strain. At one end of the disease spectrum is staphylococcal food poisoning, attributable solely to the ingestion of preformed enterotoxin; at the other end are staphylococcal bacteremia and disseminated abscesses in all organs.

Pathogenic, invasive *S aureus* produces coagulase and tends to produce a yellow pigment and to be hemolytic. Nonpathogenic, noninvasive staphylococci such as *S epidermidis* are coagulase-negative and tend to be nonhemolytic. Such organisms rarely produce suppuration but may infect orthopedic or cardiovascular prostheses or cause disease in immunosuppressed persons. They may be refractory to treatment because of the formation of biofilms.

*S saprophyticus* is typically nonpigmented, novobiocin-resistant, and nonhemolytic; it causes urinary tract infections in young women.



## Diagnostic Laboratory Tests

Specimens depending upon the localization of the infection.

### 1-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.

### 2-Culture

Specimens planted on blood agar plates. Specimens contaminated with a mixed flora can be cultured on media containing 7.5% NaCl; the salt inhibits most other normal flora but not *S aureus*. Mannitol salt agar, *S aureus* but no other staphylococci ferment mannitol.

### 3-Catalase Test

### 4-Coagulase Test

Coagulase-positive staphylococci are considered pathogenic for humans. Infections of prosthetic devices can be caused by organisms of the coagulase-negative *S epidermidis* group.

#### 5-Susceptibility Testing

Antibiotic susceptibility patterns are helpful in tracing *S aureus* infections and in determining if multiple isolates from blood cultures represent bacteremia due to the same strain.

6-Molecular typing techniques are highly discriminatory, ex. Polymerase Chain Reaction (PCR).

### **Resistance of Staphylococci to Antimicrobial Drugs**

Hospital strains of *S aureus* are often resistant to many different antibiotics by a variety of genetic mechanisms including (1) acquisition of extrachromosomal plasmids or additional genetic information in the chromosome via DNA insertion and (2) by mutations in chromosomal genes.

#### **Treatment**

In acne, lipases of staphylococci and corynebacteria liberate fatty acids from lipids and thus cause tissue irritation. Tetracyclines are used for long-term treatment.

Bacteremia, endocarditis, pneumonia, and other severe infections due to *S aureus* require prolonged intravenous therapy with a  $\beta$ -lactamase-resistant penicillin.

Alternative agents for the treatment of MRSA bacteremia and endocarditis include daptomycin, linezolid, and quinupristin-dalfopristin. *S epidermidis* infections are difficult to cure because they occur in prosthetic devices where the bacteria can sequester themselves in a biofilm. *S epidermidis* is more often resistant to antimicrobial drugs than is *S aureus*.

### ***Echinococcus granulosus* (Hydatid Cyst)**

*Echinococcus granulosus* is a dog tapeworm found only in the small intestine of dogs and other canids. The adult tapeworm ranges in length from 3 mm to 6 mm and has three proglottids ("segments"); an immature proglottid, mature proglottid and a gravid proglottid. *E. granulosus* has four suckers on its scolex ("head"), and also has a rostellum with hooks.

### **Life Cycle**

The eggs leave the final hosts and infect grazing animals. Similar to the beef and pork tapeworms, a larva hatches from the egg in an intermediate hosts, penetrates the gut, and migrates to various tissues, especially liver, spleen, muscle, and brain. Instead of a cysticercus developing, as in the case of the beef and pork tapeworms, the larva of *Echinococcus* develops into a fluid-filled cyst called a hydatid cyst. The cyst contains germinal epithelium in which thousands of future larvae (called protoscolices) develop. Inside the hydatid cyst, the protoscolices are contained within brood capsules. If the hydatid cyst ruptures, the brood capsules can spill out of the cyst, metastasize to other sites, and develop into a hydatid cyst. Thus, ingestion of a single egg can give rise to several hydatid cysts, each containing several brood capsules.

Humans can also be an intermediate host for *E. granulosus*, however this is uncommon and therefore humans are considered an aberrant intermediate host (by ingesting *Echinococcus* eggs from dog feces). Hydatid cysts which developed in viscera of man, comes to dead end.

The dog, in turn, can acquire the infection only when consuming offal containing *E. granulosus* from an infected herbivore.



## **Pathology & Pathogenesis**

Hydatid cysts can grow about 1–7 cm per year, and the symptoms depend on the location of the cysts in the body. The liver is the most common site, where compression, atrophy, portal hypertension from mechanical obstruction, and cirrhosis can occur. Extreme care must be taken when removing the cyst. If the cyst ruptures, the highly immunogenic hydatid fluid can lead to anaphylactic shock and brood capsules can metastasize to form additional hydatid cysts. When the embryo break free from the membrane and float in the fluid within the cyst, they are known as **hydatid sand**.

## **Diagnosis**

-Casoni test: an immediate hypersensitivity skin test. Antigen here is sterile, filtered hydatid fluid injected intradermal. Positive case develops a large wheal (5 cm. or more) within 30 minutes.

-Chest x-rays reveal pulmonary lesions as slightly irregular, round masses of uniform density devoid of calcification. In patients with ruptured pulmonary cysts, scolices may be demonstrated in the sputum.

-Serologic testing.

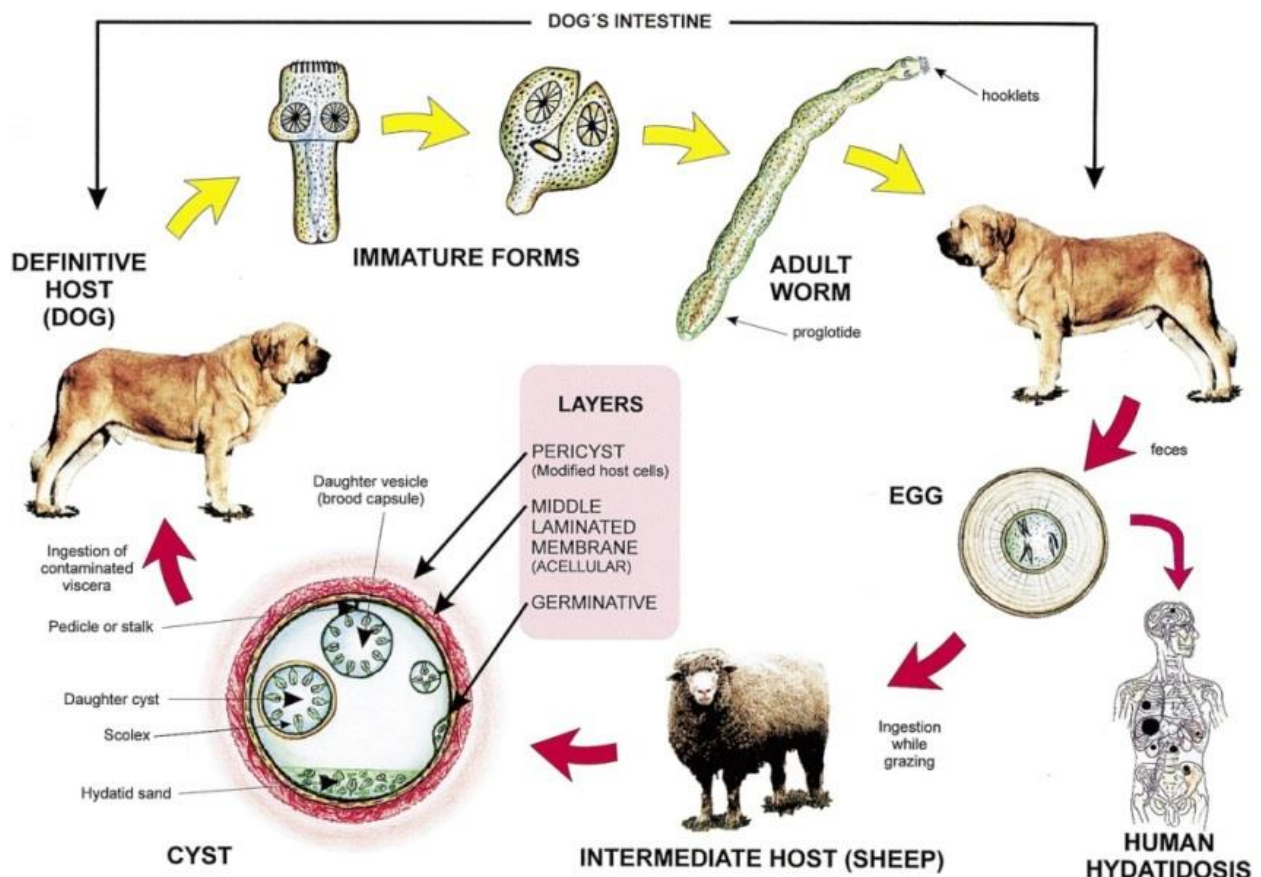
-Histological examination.

-Polymerase chain reaction assay has been shown capable of detecting very small quantities of *Echinococcus* genomic DNA in fine-needle biopsy material from patients with suspected echinococcosis.

## **Treatment and Prevention**

For years, the only definitive therapy available was surgical extirpation. Presently, it is recommended that high-dose albendazole be administered before and for several weeks after surgery and/or aspiration. Infected dogs should be wormed, and infected carcasses and offal burned or buried. Hands should be carefully washed after contact with potentially infected dogs.





**Life cycle of *Echinococcus granulosus***

### ***Echinococcus Multilocularis***

*E. multilocularis* is found primarily in subarctic and arctic regions in North America, Europe, and Asia.

### **Life Cycle**

The adult worms are found in the gut of foxes and wolves. Their larval forms find in the tissues of field mice, squirrels and voles as an intermediate hosts. Domestic dogs may acquire adult tapeworms by killing and ingesting these larval-infected rodents. Humans are accidental intermediate host, infected with larval forms through the

ingestion of eggs passed in the feces of their domestic dogs or ingestion of egg-contaminated vegetation.

### **Pathogenicity**

Unlike the larval forms of *E. granulosus*, those of *E. multilocularis* bud externally, producing proliferative, multilocular cysts called alveolar echinococcosis that slowly but progressively invade and destroy the affected organs and adjacent tissues (may be mistaken for a malignant tumor). The organ most commonly involved is the liver.

### **Diagnosis**

- Biopsy of affected organs.
- CT scan and ultrasound.
- Serologic testing.

### **Treatment**

Similar to that for *E. granulosus*.

The streptococci are gram-positive spherical bacteria that characteristically form pairs or chains during growth. 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. Most pathogenic hemolytic streptococci grow best at 37°C. Most streptococci are facultative anaerobes (Peptostreptococci are obligate anaerobes).

The streptococci are a large and heterogeneous group of bacteria and no one system suffices to classify them. Yet, understanding the classification is key to understanding their medical importance.

### **Classification of Streptococci**

The classification has been based on a series of observations over years:

#### **1-Hemolysis**

Many streptococci are able to hemolyze RBCs in vitro in varying degrees. Complete disruption of erythrocytes with clearing of the blood around the bacterial growth is called **β hemolysis**. Incomplete lysis of erythrocytes and the formation of green pigment is called **α hemolysis**. Other streptococci are nonhemolytic (**γ hemolysis**).

#### **2-Group-Specific Substance (Lancefield Classification)**

Based on the carbohydrate composition of bacterial antigens found on their cell walls of many streptococci and forms the basis of serologic

grouping into **Lancefield groups A–H** and **K–U**. The serologic specificity of the group-specific carbohydrate is determined by an amino sugar, e.g. group A streptococci, this is rhamnose-*N*-acetylglucosamine; for group B, it is rhamnose-glucosamine polysaccharide. Two groups that lack the Lancefield carbohydrate antigen: *Streptococcus pneumoniae* and Viridans streptococci.

### **3-Capsular Polysaccharides**

The antigenic specificity of the capsular polysaccharides is used to classify *S pneumoniae* into over 90 types and to type *S agalactiae*.

### **4-Biochemical Reactions**

Biochemical tests include sugar fermentation reactions, tests for the presence of enzymes, and tests for susceptibility or resistance to certain chemical agents.

Many species of streptococci, including *S. pyogenes* (group A), *S. agalactiae* (group B), and the enterococci (group D), are characterized by combinations of features: colony growth characteristics, hemolysis patterns on blood agar. The viridans streptococci can be  $\alpha$ -hemolytic or nonhemolytic and are generally speciated by biochemical reactions.

Because biochemical reactions are often unreliable, molecular genetic capabilities, such as gene sequencing, are used with these methods when identification of streptococci is required.

### ***Streptococcus pyogenes***

Most streptococci that contain the group A antigen are *S pyogenes*. It is a typical human pathogen. *S pyogenes* produces large zones of  $\beta$  hemolysis around colonies (greater than 0.5 mm in diameter), and

produce capsules inhibit phagocytosis and plays a greater role in virulence. Capsules of other streptococci (e. g, *S agalactiae* and *S pneumoniae*) are different.

The *S pyogenes* cell wall contains proteins (M, T, R antigens), carbohydrates (group-specific), and peptidoglycans. The pili project through the capsule of group A streptococci consist partly of M protein which induces antibodies that react with cardiac muscle tissue (cross-reactive antigens), and are covered with lipoteichoic acid which is important in the attachment of streptococci to epithelial cells.

### **Toxins & Enzymes**

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

#### 1-Streptokinase (Fibrinolysin)

It transforms the plasminogen of human plasma into plasmin, an active proteolytic enzyme that digests fibrin and other proteins.

#### 2-Streptodornase (streptococcal deoxyribonuclease)

It depolymerizes DNA, an antibody to DNase develops after streptococcal infections, especially after skin infections.

#### 3-Hyaluronidase

It splits hyaluronic acid, an important component of the ground substance of connective tissue (spreading factor).

#### 4-Pyrogenic Exotoxins (Erythrogenic Toxin)

Act as superantigens, associated with **streptococcal toxic shock syndrome** and **scarlet fever**.

## 5-Hemolysins

The  $\beta$ -hemolytic group A (*S. pyogenes*) elaborates two hemolysins (streptolysins). **Streptolysin O** is a protein that is hemolytically active in the reduced state, but rapidly inactivated in the presence of oxygen. It combines quantitatively with antistreptolysin O (ASO), an antibody that appears in humans following infection with any streptococci that produce streptolysin O. This antibody blocks hemolysis by streptolysin O. This phenomenon forms the basis of a quantitative test for the antibody. An ASO serum titer in excess of 160–200 units is considered abnormally high and suggests either recent infection with *S. pyogenes* or persistently high antibody levels due to an exaggerated immune response to an earlier exposure in a hyper sensitive person.

**Streptolysin S** is the agent responsible for the hemolytic zones around streptococcal colonies growing on blood agar plates. It is not antigenic, but it may be inhibited by a nonspecific inhibitor present in the sera of humans and animals.

### **Pathogenesis & Clinical Findings**

A variety of distinct disease processes are associated with *S. pyogenes* infections.

#### Diseases Attributable to Invasion by *S. pyogenes*.

- 1. Erysipelas**—If the portal of entry is the skin.
- 2. Cellulitis**—Streptococcal cellulitis is an acute, rapidly spreading infection of the skin and subcutaneous tissues.

**3. Necrotizing fasciitis (streptococcal gangrene)**—an infection of the subcutaneous tissues and fascia. There is extensive and very rapidly spreading necrosis of the skin and subcutaneous tissues.

**4. Puerperal fever**—If the streptococci enter the uterus after delivery, puerperal fever develops.

**5. Bacteremia/sepsis**— Infection of traumatic or surgical wounds with streptococci results in bacteremia, which rapidly can be fatal.

#### Diseases Attributable to Local Infection with *S pyogenes*

**1. Streptococcal sore throat-** or pharyngitis, the most common infection of *S pyogenes* adhere to the pharyngeal epithelium.

**2. Streptococcal pyoderma**—Local infection of superficial layers of skin, especially in children, is called impetigo. A clinically identical infection can be caused by *S aureus* and sometimes both *S pyogenes* and *S aureus* are present.

#### Overlapping diseases (Streptococcal Toxic Shock Syndrome, and Scarlet Fever)

Fulminant, invasive *S pyogenes* infections with streptococcal toxic shock syndrome are characterized by shock, bacteremia, respiratory failure, and multiorgan failure. Pyrogenic exotoxins A–C cause scarlet fever in association with *S. pyogenes* pharyngitis or with skin or soft tissue infection. The pharyngitis may be severe. The rash appears on the trunk after 24 hours of illness and spreads to involve the extremities.

#### Poststreptococcal Diseases (Rheumatic Fever, Glomerulonephritis)

Following an acute *S pyogenes* infection, there is a latent period of 1–4 weeks, nephritogenic M types will associate with throat infections

and glomerulonephritis. Rheumatic fever occasionally develops in patients with more severe streptococcal sore throats. The first attack of rheumatic fever usually produces only slight cardiac damage, which, however, increases with each subsequent attack. It is therefore important to protect such patients from recurrent *S pyogenes* infections by prophylactic penicillin administration.

### **Diagnostic Laboratory Tests**

Specimens: Depend upon the nature of the streptococcal infection. A throat swab, pus, or blood is obtained for culture. Serum is obtained for antibody determinations.

#### **1-Smears**

If smears of pus show streptococci but cultures fail to grow, anaerobic organisms must be suspected.

#### **2-Culture**

Specimens are cultured on blood agar plates. If anaerobes are suspected, suitable media must also be inoculated. Incubation in 10% CO<sub>2</sub> often speeds hemolysis.

#### **3-Serologic Tests**

A rise in the titer of antibodies (as ASO) to many group A streptococcal antigens can be estimated, particularly in respiratory disease; anti-DNase and antihyaluronidase, particularly in skin infections; and others.

### **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.

### ***Streptococcus agalactiae* (group B streptococci)**

They are part of the normal vaginal flora and lower gastrointestinal tract in 5–25% of women. They typically are  $\beta$ -hemolytic.

Group B streptococcal infection during the first month of life may present as fulminant sepsis, meningitis, or respiratory distress syndrome. Intravenous ampicillin given to mothers, who carry group B streptococci and are in labor, prevents colonization of their infants and group B streptococcal disease.

Two expanding populations, namely the elderly and immunocompromised hosts, are most at risk for invasive disease. Bacteremia, skin and soft tissue infections, respiratory infections, and genitourinary infections are the major clinical manifestations.

### **Group D Streptococci**

All Group D streptococci are nonhemolytic, grow in the presence of bile and hydrolyze esculin (bile-esculin positive). They are part of the normal enteric flora.

### **Viridans Streptococci**

The viridans streptococci are the most prevalent members of the normal flora of the upper respiratory tract and are important for the healthy state of the mucous membranes there. They may reach the bloodstream as a result of trauma and are a principal cause of endocarditis on abnormal heart valves. Some viridans streptococci (eg, *S mutans*)

synthesize large polysaccharides such as dextrans or levans from sucrose and contribute importantly to the genesis of dental caries.

In the course of bacteremia, viridans streptococci, pneumococci, or enterococci may settle on normal or previously deformed heart valves, producing **acute endocarditis**. Rapid destruction of the valves frequently leads to fatal cardiac failure in days or weeks unless a prosthesis can be inserted during antimicrobial therapy.

**Subacute endocarditis** is most frequently due to members of the normal flora of the respiratory or intestinal tract that have accidentally reached the blood. After dental extraction, at least 30% of patients have viridans streptococcal bacteremia.

$\alpha$ -Hemolytic streptococci and enterococci vary in their susceptibility to antimicrobial agents, antibiotic susceptibility tests are useful to determine which drugs may be used for optimal therapy.

### ***Streptococcus pneumoniae***

The pneumococci (*S pneumoniae*) are diplococci, often lancet-shaped or arranged in chains. Pneumococci form small round colonies,  $\alpha$ -hemolytic on blood agar. Growth is enhanced by 5–10% CO<sub>2</sub>, possessing a capsule of polysaccharide that permits typing with specific antisera. Pneumococci are normal inhabitants of the upper respiratory tract of 5–40% of humans and can cause pneumonia, sinusitis, otitis, bronchitis, bacteremia, meningitis, and other infectious processes.

With age, the organisms rapidly become gram-negative and tend to lyse spontaneously. On solid media, the growth of pneumococci is inhibited

around a disk of Optochin; viridans streptococci are not inhibited by Optochin.

### **Antigenic Structure**

The capsular polysaccharide is immunologically distinct for each of the more than 90 types. Pneumococcal isolates that produce large amounts of capsules produce large mucoid colonies.

### **Quellung Reaction**

is a biochemical reaction in which antibodies bind to the bacterial capsule of *Strep. pneumoniae*, *Klebsiella pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Escherichia coli*, and *Salmonella*. The antibody reaction allows these species to be visualized under a microscope. If the reaction is positive, the capsule becomes opaque and appears to enlarge. When pneumococci of a certain type is mixed with specific antipolysaccharide serum on a microscope slide, the capsule swells markedly, and the organisms agglutinate by cross-linking of the antibodies. This reaction is useful for rapid identification and for typing of the organisms, either in sputum or in cultures. The polyvalent antiserum, which contains antibody to all of the types ("omniserum"), is a good reagent for rapid microscopic determination of whether or not pneumococci are present in fresh sputum.

### **Pathogenesis**

Types of Pneumococci: In adults, types 1–8 are responsible for about 75% of cases of pneumococcal pneumonia and for more than half of all

fatalities in pneumococcal bacteremia; in children, types 6, 14, 19, and 23 are frequent causes.

Production of Disease: Pneumococci produce disease through their ability to multiply in the tissues. They produce no toxins of significance. The virulence of the organism is a function of its capsule, which prevents or delays ingestion by phagocytes. A serum that contains antibodies against the type-specific polysaccharide protects against infection. Animals or humans immunized with a given type of pneumococcal polysaccharide are subsequently immune to that type of pneumococcus and possess precipitating and opsonizing antibodies for that type of polysaccharide.

### **Clinical Findings**

The onset of pneumococcal pneumonia is usually sudden, with fever, chills, and sharp pleural pain. The sputum is similar to the alveolar exudate, being characteristically bloody or rusty colored. Early in the disease, when the fever is high, bacteremia is present in 10–20% of cases. With antimicrobial therapy, the illness is usually terminated promptly; if drugs are given early, the development of consolidation is interrupted.

From the respiratory tract, pneumococci may reach other sites. The sinuses and middle ear are most frequently involved. Infection sometimes extends from the mastoid to the meninges. Bacteremia from pneumonia has a triad of severe complications: meningitis, endocarditis, and septic arthritis. With the early use of chemotherapy, acute pneumococcal endocarditis and arthritis have become rare.

## **Diagnostic Laboratory Tests**

Blood for culture; CSF and sputum are collected for demonstration of pneumococci by smear and culture. Serum antibody tests are impractical.

Sputum may be examined by Gram-stained film and capsule swelling (the quellung reaction). The culture is created by sputum cultured on blood agar and incubated in CO<sub>2</sub> or a candle jar. A blood culture is also taken.

## **Treatment**

Since pneumococci are sensitive to many antimicrobial drugs, early treatment usually results in rapid recovery, and antibody response seems to play a much diminished role. Penicillin G is the drug of choice. High-dose penicillin G appears to be effective in treating pneumonia caused by pneumococci but would not be effective in treatment of meningitis due to the same strains. Resistance to tetracycline and erythromycin may occur. Pneumococci remain susceptible to vancomycin.

**Definitions**

**Sterilization** is complete killing, or removal, of all living organisms from a particular location or material. It can be accomplished by incineration, certain gases, exposure to ionizing radiation, etc.....

**Disinfection** is the destruction of pathogenic microorganisms by processes that fail to meet the criteria for sterilization. The term is most commonly applied to the use of liquid chemical agents known as disinfectants, which usually have some degree of selectivity. Bacterial spores, organisms with waxy coats (eg, mycobacteria), and some viruses may show considerable resistance to the common disinfectants.

**Antiseptics** are disinfectant agents that can be used on body surfaces such as the skin to reduce the numbers of normal flora and pathogenic contaminants. They have lower toxicity than disinfectants used environmentally, but are usually less active in killing vegetative organisms. **Sanitization** is a less precise term with a meaning somewhere between disinfection and cleanliness. It is used primarily in housekeeping and food preparation contexts.

**Death/killing** it is a loss of ability to multiply under any known conditions. This is complicated by the fact that organisms that appear to be irreversibly inactivated may sometimes recover when appropriately treated. For example, ultraviolet irradiation of bacteria can result in the formation of thymine dimers in the DNA with loss of ability to replicate. A period of exposure to visible light may then activate an enzyme that breaks the dimers and restores viability by a process known as photoreactivation. Mechanisms also exist for repair of the damage without light. Such considerations are of great significance in the preparation of safe vaccines from inactivated virulent organisms.

**Asepsis** describes processes designed to prevent microorganisms from reaching a protected environment. It is applied in many procedures

used in the operating room, in the preparation of therapeutic agents, and in technical manipulations in the microbiology laboratory.

The various modes of sterilization are summarized in a Table:

METHOD	ACTIVITY LEVEL	SPECTRUM	USES/COMMENTS
<b>Heat</b>			
Autoclave	Sterilizing	All	General
Boiling	High	Most pathogens, some spores	General
Pasteurization	Intermediate	Vegetative bacteria	Beverages, plastic hospital equipment
<b>Ethylene oxide gas</b>	Sterilizing	All	Potentially explosive; aeration required
<b>Radiation</b>			
Ultraviolet	Sterilizing	All	Poor penetration
Ionizing	Sterilizing	All	General, food
<b>Chemicals</b>			
Alcohol	Intermediate	Vegetative bacteria, fungi, some viruses	
Hydrogen peroxide	High	Viruses, vegetative bacteria, fungi	Contact lenses; inactivated by organic matter
Chlorine	High	Viruses, vegetative bacteria, fungi	Water; inactivated by organic matter
Iodophors	Intermediate	Viruses, vegetative bacteria, <sup>a</sup> fungi	Skin disinfection; inactivated by organic matter
Phenolics	Intermediate	Some viruses, vegetative bacteria, fungi	Handwashing
Glutaraldehyde	High	All	Endoscopes, other equipment
Quaternary ammonium compounds	Low	Most bacteria and fungi, lipophilic viruses	General cleaning; inactivated by organic matter

## **Microbial Killing**

Killing of bacteria by heat, radiation, or chemicals is usually exponential with time; that is, a fixed proportion of survivors are killed during each time increment. Thus, if 90% of a population of bacteria are killed during each 5 minutes of exposure to a weak solution of a disinfectant, a starting population of  $10^6$ /mL is reduced to  $10^5$ /mL after 5 minutes, to  $10^3$ /mL after 15 minutes, and theoretically to 1 organism ( $10^0$ )/mL after 30 minutes. The killing process is influenced by the nature of the organism, lethal agent, concentration (in the case of disinfectants), and temperature.

## **Heat**

The simplest method of sterilization is to expose the surface to be sterilized to a naked flame, as is done with the wire loop used in microbiology laboratories. It can be used equally effectively for emergency sterilization of a knife blade or a needle.

Carbonization of organic material and destruction of microorganisms, including spores, occur after exposure to dry heat of  $160^\circ\text{C}$  for 2 hours in a sterilizing oven. This method is applicable to metals, glassware, and some heat-resistant oils and waxes that are immiscible in water and, therefore, cannot be sterilized in the autoclave. A major use of the dry heat sterilizing oven is in preparation of laboratory glassware.

Moist heat in the form of water or steam is far more rapid and effective in sterilization than dry heat because reactive water molecules denature protein irreversibly by disrupting hydrogen bonds between peptide groups at relatively low temperatures. Most vegetative bacteria of importance in human disease are killed within a few minutes at  $70^\circ\text{C}$  or less, although many bacterial spores can resist boiling for prolonged periods.

For applications requiring sterility, the use of boiling water has been replaced by the autoclave, which, when properly used, ensures sterility by killing all forms of microorganisms. Autoclaves are usually operated at  $121^\circ\text{C}$ , which is achieved with a pressure of 15 pounds per square inch. Under these conditions, spores directly exposed are killed in less



than 5 minutes, although the normal sterilization time is 10 to 15 minutes to account for variation in the ability of steam to penetrate different materials and to allow a wide margin of safety. For example, the spores of *Clostridium botulinum*, the cause of botulism, may survive 5 hours of boiling, but can be killed in 4 minutes at 121°C in the autoclave. Autoclaves can thus be used for sterilizing any materials that are not damaged by heat and moisture, such as heat-stable liquids, swabs, most instruments and culture media.

### **Gas**

A number of articles, particularly certain plastics and lensed instruments that are damaged or destroyed by autoclaving, can be sterilized with gases. **Ethylene oxide** is an inflammable and potentially explosive gas. It is an alkylating agent that inactivates microorganisms by replacing labile hydrogen atoms on hydroxyl, carboxy, or sulfhydryl groups, particularly of guanine and adenine in DNA. Ethylene oxide sterilizers resemble autoclaves. Ethylene oxide is an effective sterilizing agent for heat-labile devices such as artificial heart valves that cannot be treated at the temperature of the autoclave. Exposure times are usually about 4 to 6 hours and must be followed by a prolonged period of aeration to allow the gas to diffuse out of substances that have absorbed it. Aeration is essential, because absorbed gas can cause damage to tissues or skin. Ethylene oxide is a mutagen, and special precautions are now taken to ensure that it is properly vented outside of working spaces.

Other alkylating agents such as **formaldehyde** vapor can be used without pressure to decontaminate larger areas such as rooms, and oxidizing agents (hydrogen peroxide, ozone) have selective use.

### **Ultraviolet Light and Ionizing Radiation**

Ultraviolet (UV) light in the wavelength range of 240 to 280 nm is absorbed by nucleic acids and causes genetic damage, including the formation of the thymine dimers discussed previously. The practical value of UV sterilization is limited by its poor ability to penetrate. Apart from the experimental use of UV light as a mutagen, its main

application has been in irradiation of air in the vicinity of critical hospital sites and as an aid in the decontamination of laboratory facilities used for handling particularly hazardous organisms. In these situations, single exposed organisms are rapidly inactivated. It must be remembered that UV light can cause skin and eye damage, and workers exposed to it must be appropriately protected.

**Ionizing radiation** carries far greater energy than UV light. It causes direct damage to DNA and produces toxic free radicals and hydrogen peroxide from water within the microbial cells. Cathode rays and gamma rays from cobalt-60 are widely used in industrial processes, including the sterilization of many disposable surgical supplies such as gloves, plastic syringes, specimen containers, some foodstuffs, and the like, because they can be packaged before exposure to the penetrating radiation. Recent food-borne outbreaks (*Escherichia coli*) and bioterrorism (anthrax) have increased the use of ionizing radiation.

## **Disinfection**

### **Physical Methods**

#### **Filtration**

Both live and dead microorganisms can be removed from liquids by filtration. Membrane filters, usually composed of cellulose esters (eg, cellulose acetate), are available commercially with pore sizes of 0.005 to 1  $\mu\text{m}$ . For removal of bacteria, a pore size of 0.2  $\mu\text{m}$  is effective because filters act not only mechanically but by electrostatic adsorption of particles to their surface. Filtration is used for disinfection of large volumes of fluid, especially fluid containing heat-labile components such as serum. For microorganisms larger than the pore size, filtration "sterilizes" these liquids. It is not considered effective for removing viruses.

#### **Pasteurization**

Pasteurization involves exposure of liquids to temperatures in the range 55° to 75°C to remove all vegetative bacteria of significance in human disease. Spores are unaffected by the pasteurization process. Pasteurization is used commercially to render milk safe and to extend

its storage quality. With the outbreaks of infection due to contamination with enterohemorrhagic *E coli*, this has been extended to fruit drinks. Pasteurization in water at 70°C for 30 minutes has been effective and inexpensive when used to render inhalation therapy equipment free of organisms that may otherwise multiply in mucus and humidifying water.

### **Microwaves**

The use of microwaves in the form of microwave ovens or specially designed units is another method of disinfection. These systems are not under pressure, but they can achieve temperatures near boiling if moisture is present. In some situations, they are being used as a practical alternative to incineration for disinfection of hospital waste. These procedures cannot be considered sterilization only because heat-resistant spores may survive the process.

### **Chemical Methods**

Given access and sufficient time, chemical disinfectants cause the death of pathogenic vegetative bacteria. Most of these substances are general protoplasmic poisons and are not used in the treatment of infections other than very superficial lesions, having been replaced by antimicrobics. Some disinfectants such as the quaternary ammonium compounds, alcohol, and the iodophors reduce the superficial flora and can eliminate contaminating pathogenic bacteria from the skin surface. Other agents such as the phenolics are valuable only for treating inanimate surfaces or for rendering contaminated materials safe. All are bound and inactivated to varying degrees by protein and dirt, and they lose considerable activity when applied to other than clean surfaces.

#### **-Alcohol**

The alcohols are protein denaturants that rapidly kill vegetative bacteria when applied as aqueous solutions in the range of 70% to 95% alcohol. They are inactive against bacterial spores and many viruses. Solutions of 100% alcohol dehydrate organisms rapidly but fail to kill, because the lethal process requires water molecules. Ethanol (70-90%) and

isopropyl alcohol (90-95%) are widely used as skin decontaminants before simple invasive procedures such as venipuncture.

**-Iodine** is an effective disinfectant that acts by iodinating or oxidizing essential components of the microbial cell. Its original use was as a tincture of 2% iodine in 50% alcohol, which kills more rapidly and effectively than alcohol alone. They are widely used in preparation of skin before surgery. This preparation has the disadvantage of sometimes causing hypersensitivity reactions and of staining materials with which it comes in contact.

**-Chlorine** is a highly effective oxidizing agent, which accounts for its lethality to microbes. In concentrations of less than one part per million, chlorine is lethal within seconds to most vegetative bacteria, and it inactivates most viruses; this efficacy accounts for its use in rendering supplies of drinking water safe and in chlorination of water in swimming pools. Chlorine reacts rapidly with protein and many other organic compounds, and its activity is lost quickly in the presence of organic material. It is usually applied as a 5% solution called hypochlorite.

The use of chlorination to disinfect water supplies has proved insufficient in some hospitals because of the relative resistance of *Legionella pneumophila* to the usual concentrations of chlorine. Some institutions have been forced to augment chlorination with systems that add copper and silver ions to the water.

### **-Hydrogen Peroxide**

is a powerful oxidizing agent that attacks membrane lipids and other cell components. Although it acts rapidly against many bacteria and viruses, it kills bacteria that produce catalase and spores less rapidly. Hydrogen peroxide has been useful in disinfecting items such as contact lenses, which are not susceptible to its corrosive effect.

### **-Surface-Active Compounds**

**Surfactants** are compounds with hydrophobic and hydrophilic groups that attach to and solubilize various compounds or alter their properties. Anionic detergents such as soaps are highly effective cleansers, but

have little direct antibacterial effect, probably because their charge is similar to that of most microorganisms. Cationic detergents, particularly the **quaternary ammonium compounds** ("quats") such as benzalkonium chloride, are highly bactericidal in the absence of contaminating organic matter. Their hydrophobic and lipophilic groups react with the lipid of the cell membrane of the bacteria, alter the membrane's surface properties and its permeability, and lead to loss of essential cell components and death. They are inactive against spores and most viruses.

### **-Phenolics**

**Phenol** is a potent protein denaturant and bactericidal agent. Substitutions in the ring structure of phenol have substantially improved activity and have provided a range of phenols and cresols that are the most effective environmental decontaminants available for use in hospital hygiene. Concern about their release into the environment in hospital waste and sewage has created some pressure to limit their use. This is another of the classic environmental dilemmas of our society: a compound that reduces the risk of disease for one group may raise it for another.

Chlorhexidine a routine hand and skin disinfectant and for other topical applications. It has great bactericidal activity. It acts by altering membrane permeability of both Gram-positive and Gram-negative bacteria. It is cationic and thus its action is neutralized by soaps and anionic detergents.

### **-Glutaraldehyde and Formaldehyde**

are alkylating agents highly lethal to essentially all microorganisms. Formaldehyde gas is irritative, allergenic, and unpleasant, properties that limit its use as a solution or gas. Glutaraldehyde is an effective high-level disinfecting agent for apparatus that cannot be heat treated, such as some lensed instruments and equipment for respiratory therapy. Formaldehyde vapor, an effective environmental decontaminant under conditions of high humidity, is sometimes used to decontaminate laboratory rooms that have been accidentally and extensively

contaminated with pathogenic bacteria, including those such as the anthrax bacillus that form resistant spores.

### **Infection Control and Nosocomial Infections**

Some risk of infection exists in all health care settings. Hospitalized patients are particularly vulnerable, and the hospital environment is complex. "Nosocomial" is a medical term for "hospital-associated." Nosocomial infections are complications that arise during hospitalizations. The morbidity, mortality, and costs associated with these infections are preventable to a substantial degree. The purpose of hospital infection control is prevention of nosocomial infections by application of epidemiologic concepts and methods.

The infectious agents responsible for nosocomial infections arise from various sources, including patients' own normal flora. In addition to any immunocompromising disease or therapy, the hospital may impose additional risks by treatments that breach the normal defense barriers. Surgery, urinary or intravenous catheters, and invasive diagnostic procedures all may provide normal flora with access to usually sterile sites. Infections in which the source of organisms is the hospital rather than the patient include those derived from hospital personnel, the environment, and medical equipment.

Aerobic, nonmotile bacteria (except for the species *Mycobacterium marinum*, which has been shown to be motile within macrophages) they are characteristically acid fast and most do not form endospores. The genus includes pathogens known to cause serious diseases in mammals, including tuberculosis (*M. tuberculosis*) and Leprosy or Hansen's disease (*M. leprae*). *M. bovis* in cattle can transmit to human by ingestion of infected milk.

### ***Mycobacterium tuberculosis***

First discovered by Robert Koch, it has an unusual, waxy coating on its cell surface due to the presence of mycolic acid which account for 60% of cell wall weight. It is likely responsible for this resistance and is a key virulence factor, which makes the cells impervious to Gram staining. The Ziehl-Neelsen stain, or acid-fast stain, is used instead. Mycolic acid prevent attack of the mycobacteria by cationic proteins, lysozyme, and oxygen radicals in the phagocytic granule and resistance to many antibiotics. They also protect extracellular mycobacteria from complement deposition in serum. The physiology of *M. tuberculosis* (TB) is highly aerobic and requires high levels of oxygen. Humans are the only reservoir for the bacterium, it infects the lungs. The bacterium is a facultative intracellular, usually of macrophages, and has a slow generation time, 15-20 hours.

### **Pathogenesis and Lesion Development**

Infections usually begin by inhalation of aerosol droplets containing tubercle bacilli directly expectorated from an individual with “open”

pulmonary disease. The infectious dose for a person is reported to be between 1 and 200 bacilli. The bacilli travel to the alveoli, where they are rapidly phagocytosed by alveolar macrophages. These macrophages are stimulated to produce proinflammatory cytokines and chemokines, driving the recruitment of more leukocytes to the site of infection. Neutrophils and monocytes arrive first, phagocytose additional bacteria, secrete more cytokines and chemokines, and begin to organize the early granuloma then migrate to regional lymph nodes to present mycobacterial antigens to lymphocytes.

TB adapt to this environment by preferentially using fatty acids in their metabolism, slowing down active replication, and increasing cell wall thickness, entering a so-called dormant state.

The lesion at the primary site of implantation is termed the Ghon focus. Hematogenous dissemination within the lung or to other organs can also occur during the early stage of this disease. Interestingly, the upper lung lobes of the human favor bacillary growth due to higher oxygen pressure and delayed immune responses.

This ability to establish a chronic asymptomatic infection, followed by reactivation and transmission years later to new uninfected hosts, lends to the tremendous success of TB as a pathogen.

Although symptoms of primary TB in most cases are generally subtle and easily overlooked, secondary cases are characterized by localized symptoms, such as coughing, hemoptysis, and pleuritic pain, as well as the generalized symptoms of fever, anorexia, night sweats, and



cachexia. If the host is able to reestablish immune control over the disease, recovery is also possible.

### **Diagnostic methods for tuberculosis**

-Sputum: smears and cultures should be done for acid-fast bacilli if the patient is producing sputum. Alternative sampling in patients incapable of producing a sputum include; laryngeal swab, bronchoscopy (with bronchoalveolar lavage, bronchial washings, and/or transbronchial biopsy).

- Culture: TB grows in Lowenstein Jensen medium, is an egg based media with addition of salts, 5 % glycerol, Malachite green & penicillin to keep contaminants from outgrowing the organism. Because of its slow growth, it takes 4-6 weeks before small buff colored colonies are visible on the medium.

-PCR: Other mycobacteria are also acid-fast. If the smear is positive, PCR or gene probe tests can distinguish TB from other mycobacteria.

-Radiography: Chest X-ray and CT (computed tomography).

-Immunological test: as Tuberculin skin test.

### **Treatment for TB Disease**

TB bacteria become active (multiplying in the body) if the immune system can't stop them from growing. When TB bacteria are active, this is called TB disease. TB disease will make a person sick. People with TB disease may spread the bacteria to people with whom they spend many hours.

TB disease can be treated by taking several drugs for 6 to 9 months. Of the approved drugs, the first-line anti-TB agents that form the core of treatment regimens include:

isoniazid (INH), rifampin (RIF), ethambutol (EMB), pyrazinamide (PZA).

## *Mycobacterium leprae*

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In size, shape, and its thick waxy coating, *M. leprae* is closely resembles *M. tuberculosis*. The generation time 12-13 days, culture takes several weeks to mature.

### **Pathogenesis**

The incubation period of *M. leprae* can range between 9 months and 20 years. It replicates intracellularly inside histocytes and nerve cells and has two forms. A person's immune response to the disease determines their forms of leprosy. One form is tuberculoid, which induces a cell-mediated response that limits its growth. Through this form *M. leprae* multiplies at the site of entry, usually the skin, invading and colonizing Schwann cells. The microbe then induces T-helper lymphocytes, epitheloid cells, and giant cell infiltration of the skin, causing infected individuals to exhibit large flattened patches with raised and elevated red edges on their skin. These patches have dry, pale, hairless centers, accompanied by a loss of sensation on the skin. The macule at the cutaneous site of entry and the loss of pain sensation are key clinical indications that an individual has a tuberculoid form of leprosy.

The second form of leprosy is the lepromatous form. This form of the microbe proliferates within the macrophages at the site of entry. It also grows within the epithelial tissues of the face and ear lobes. The suppressor T-cells that are induced are numerous, however the epithelioid and giant cells are rare or absent. With cell-mediated immunity impaired, large numbers of *M. leprae* appear in the macrophages and the infected patients develop papules at the entry site, marked by a folding of the skin. Extensive penetration of this microbe may lead to severe body damage; for example the loss of bones, fingers, and toes.

### **Diagnosis**

- Lepromin skin test can be used to differentiate the two different forms.
- Acid fast staining for skin lesion biopsy
- Serology
- No culture

### **Treatment**

Antibiotics that destroy *M. leprae* bacilli include: dapsone, rifampin, clofazamine, fluoroquinolones, macrolides, and minocycline.

A preventative measure of *M. leprae* is to avoid close contact with infectious people who are untreated. An armadillo can also carry and transmit the disease to humans.

*H. pylori* is a Gram-negative, microaerophilic bacterium found in the stomach, and may be present in other parts of the body, such as the eye. It was identified in 1982 by Australian scientists Barry Marshall and Robin Warren, who found that it was present in a person with chronic gastritis and gastric ulcers, conditions not previously believed to have a microbial cause. It is also linked to the development of duodenal ulcers and stomach cancer. However, over 80% of individuals infected with the bacterium are asymptomatic.

*H. pylori*'s helical shape (from which the genus name is derived) is thought to have evolved to penetrate the mucoid lining of the stomach. It is about 3  $\mu\text{m}$  long with a diameter of about 0.5  $\mu\text{m}$ , has four to six lophotrichous flagella; all gastric and enterohepatic *Helicobacter* species are highly motile owing to flagella.

It is microaerophilic; that is, it requires oxygen, but at lower concentration than is found in the atmosphere. It contains a hydrogenase which can be used to obtain energy by oxidizing molecular hydrogen ( $\text{H}_2$ ) produced by intestinal bacteria. It produces oxidase, catalase, and urease. It is capable of forming biofilms and can convert from spiral to a possibly viable but nonculturable coccoid form, both likely to favor its survival and be factors in the epidemiology of the bacterium.

*H. pylori* possesses five major outer membrane protein families. The largest family includes known and putative adhesins. The other four

families are porins, iron transporters, flagellum-associated proteins, and proteins of unknown function. Like other typical Gram-negative bacteria, the outer membrane of *H. pylori* consists of phospholipids and lipopolysaccharide (LPS).

## **Pathophysiology:**

### **-Adaptation to the stomach's acidic environment**

To avoid the acidic environment of the interior of the stomach (lumen), *H. pylori* uses its flagella to burrow into the mucus lining of the stomach to reach the epithelial cells underneath, where the pH is more neutral, this also keeps the bacteria from being swept away into the lumen. Also neutralizes the acid in its environment by producing large amounts of urease, which breaks down the urea present in the stomach to carbon dioxide and ammonia. The ammonia, which is basic, then neutralizes stomach acid.

It adheres to the epithelial cells by producing adhesins, which bind to lipids and carbohydrates in the epithelial cell membrane.

### **-Inflammation, gastritis, and ulcer**

*H. pylori* harms the stomach and duodenal linings by several mechanisms. The ammonia produced to regulate pH is toxic to epithelial cells, as are biochemicals produced by *H. pylori* such as proteases, vacuolating cytotoxin A (VacA) [It is a toxin, present in every *H. pylori* strain, encoded by a gene. It forms vacuoles in host cells. It also turns off the infection fighting WBCs in the stomach, diminishing the immune response to *H. pylori* and this damages

epithelial cells, disrupts tight junctions and causes apoptosis], and certain phospholipases. Cytotoxin associated gene CagA can also cause inflammation and is potentially a carcinogen.

Colonization of the stomach by *H. pylori* can result in chronic gastritis and an inflammation of the stomach lining, at the site of infection then causing ulcers.

### **-Cancer**

Two related mechanisms by which *H. pylori* could promote cancer are under investigation. One mechanism involves the enhanced production of free radicals near *H. pylori* and an increased rate of host cell mutation. The strain of *H. pylori* to which a person is exposed may influence the risk of developing gastric cancer. Strains of *H. pylori* that produce high levels of two proteins, VacA and the CagA, appear to cause greater tissue damage than those that produce lower levels or that lack those genes completely. These proteins are directly toxic to cells lining of the stomach and signal strongly to the immune system that an invasion is underway. As a result of the bacterial presence, neutrophils and macrophages set up residence in the tissue to fight the bacteria assault.

### **Diagnosis**

\*Invasive tests, based on gastric specimens (Endoscopy):

-Rapid urease test: gastric biopsy can be placed into urea containing medium with color indicator, if *H.pylori* is present the urease rapidly splits urea then shift in PH yields a color change in the medium.

-Histology: histopathology.

-Culture: *H.pylori* grows on skirrows medium with vancomycin, polymyxin and trimethoprim.

\*Noninvasive tests: based on peripheral samples:

-Serologic (Abs) detection.

-Stool antigen test.

-Urea breath test (C<sup>14</sup> Urea, C<sup>13</sup> Urea): in this test C<sup>14</sup> Urea, C<sup>13</sup> Urea labeled urea is ingested by patient, if *H.pylori* is present the urease activity generates labeled CO<sub>2</sub> that can be detected in patient's exhaled breath.

## **Treatment**

A combination of two different antibiotics, together with another drug can reduce stomach acid. Lowering stomach acid helps the antibiotics work more effectively. This treatment is sometimes referred to as triple therapy.

Some of the drugs that are used in a triple therapy treatment include:

- clarithromycin
  - proton-pump inhibitors (PPI), such as omeprazole, esomeprazole (Nexium).
  - metronidazole (for seven to 14 days)
  - amoxicillin (for seven to 14 days)
-

## ***Salmonella***

Is a genus of rod-shaped (bacillus), non-spore-forming, motile with peritrichous flagella, facultative anaerobes gram-negative bacteria of the Enterobacteriaceae family. Most common species of *Salmonella* isolated and known to cause infections in humans *Salmonella enterica*, is further subdivided into 6 subspecies with about 2500 serovars. *S. enterica* serovars *S. enteritidis*, *S. typhimurium* referred to as nontyphoidal *Salmonella*, cause gastroenteritis (Salmonellosis), whereas the serovars *S. typhi*, *S. paratyphi A*, *S. paratyphi B*, *S. paratyphi C* referred to as typhoidal *Salmonella*, cause enteric typhoid fever.

### **Detection, culture and growth conditions**

Most subspecies of *Salmonella* produce hydrogen sulfide, which can readily be detected by growing them on media containing ferrous sulfate, such as is used in the triple sugar iron test.

*Salmonella* can be found in the digestive tracts of humans and animals, especially reptiles. *Salmonella* on the skin of reptiles or amphibians can be passed to people who handle the animals. Food and water can also be contaminated with the bacteria if it comes in contact with the feces of infected people or animals.

Antigenic Types of *Salmonella* species characterized by O, H, and Vi antigens (Vi antigen is a superficial antigen overlying the O antigen) using polyvalent and specific antisera (Widal test).

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## **Diagnosis**

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- Biochemical tests. Urease test is negative, Oxidase test is negative, Indole test is negative.
- Culture method: Feces, blood, or other specimens should be plated on several nonselective and selective agar media (blood, MacConkey, eosin-methylene blue, bismuth sulfite, Salmonella-Shigella, and brilliant green agars) as well as into enrichment broth such as selenite or tetrathionate. Any growth in enrichment broth is subsequently subcultured onto the various agars. The biochemical reactions of suspicious colonies are then determined on triple sugar iron agar and lysine-iron agar, and a presumptive identification is made.
- Agglutination Test for Salmonella species.
- Phage typing.
- Molecular methods (PCR).

## **Clinical Manifestations and Pathogenesis**

Salmonellosis ranges clinically from the common Salmonella gastroenteritis (diarrhea, abdominal cramps, and fever) to enteric fevers (including typhoid fever) which are life-threatening febrile systemic illness requiring prompt antibiotic therapy. Focal infections and an asymptomatic carrier state occur. The most common form of salmonellosis is a self-limited, uncomplicated gastroenteritis.

Most non-typhoidal salmonellae enter the body when contaminated food is ingested. Person-to-person spread of salmonellae also occurs. To be fully pathogenic, salmonellae must possess virulence factors. These include:

(1) the ability to invade cells, (2) a complete lipopolysaccharide coat, (3) the ability to replicate intracellularly, and (4) possibly the elaboration of toxin(s). After ingestion, the organisms colonize the ileum and colon, invade the intestinal epithelium, and proliferate within the epithelium and lymphoid follicles. Invasion occurs by the organism triggering mechanism that involves cytoskeletal alterations resulting in the formation of actin-rich membrane ruffles that engulf the pathogen. Attachment and invasion are under distinct genetic control and involve multiple genes in both chromosomes and plasmids. After invading the epithelium, the organisms multiply intracellularly and then spread to mesenteric lymph nodes and throughout the body via the systemic circulation; they are taken up by the reticuloendothelial cells. However, depending on the serotype and the effectiveness of the host defenses against that serotype, some organisms may infect the liver, spleen, gallbladder, bones, meninges, and other organs. Fortunately, most serovars are killed promptly in extraintestinal sites, and the most common human *Salmonella* infection, gastroenteritis, remains confined to the intestine.

After invading the intestine, most salmonellae induce an acute inflammatory response, which can cause ulceration. They may elaborate cytotoxins that inhibit protein synthesis.

However, invasion of the mucosa causes the epithelial cells to synthesize and release various proinflammatory cytokines. These evoke an acute inflammatory response and may also be responsible for damage to the intestine. Because of the intestinal inflammatory

reaction, symptoms of inflammation such as fever, chills, abdominal pain, leukocytosis, and diarrhea are common. The stools may contain polymorphonuclear leukocytes, blood, and mucus.

Only strains that penetrate the intestinal mucosa are associated with the appearance of an acute inflammatory reaction and diarrhea; the diarrhea is due to secretion of fluid and electrolytes by the small and large intestines. Systemic spread of the organisms can occur, giving rise to enteric fever. Invasion of the intestinal mucosa is followed by activation of mucosal adenylate cyclase; the resultant increase in cyclic AMP induces secretion, it may involve local production of prostaglandins or other components of the inflammatory reaction. In addition, *Salmonella* strains elaborate one or more enterotoxin-like substances which may stimulate intestinal secretion.

### **Host Defenses**

Both nonspecific and specific host defenses are active. Non-specific defenses consist of gastric acidity, intestinal mucus, intestinal motility (peristalsis), lactoferrin, and lysozyme. Specific defenses consist of mucosal and systemic antibodies and genetic resistance to invasion. Various factors affect susceptibility.

### **Control**

*Salmonellae* are difficult to eradicate from the environment. However, because the major reservoir for human infection is poultry and livestock, reducing the number of salmonellae harbored in these animals would significantly reduce human exposure. Other helpful measures include changing animal slaughtering practices to reduce

cross-contamination of animal carcasses; protecting processed foods from contamination; providing training in hygienic practices for all food-handling personnel in slaughterhouses, food processing plants, and restaurants; cooking and refrigerating foods adequately in food processing plants, restaurants, and homes; and expanding of governmental enteric disease surveillance programs.

Vaccines are available for typhoid fever and are partially effective, especially in children. No vaccines are available for non-typhoidal salmonellosis.

### **Treatment**

General salmonellosis treatment measures include replacing fluid loss by oral and intravenous routes, and controlling pain, nausea, and vomiting. Specific therapy consists of antibiotic administration. Typhoid fever and enteric fevers should be treated with antibiotics. Antibiotic therapy of non-typhoidal salmonellosis should be reserved for the septicemic, enteric fever, and focal infection syndromes.

The genus contains two important pathogenic species:

*Neisseria meningitidis* (meningococcus): causes cerebrospinal meningitis in man. *Neisseria gonorrhoeae* (gonococcus): causes gonorrhoea in man. Also other are commensals species such as: *Moraxella catarrhalis* & *Neisseria sicca*.

Microscopical appearance:

- Gram-ve diplococci in pairs
- The cocci are kidney-shaped with flat or concave opposing sides.
- Non-motile, non-spore forming
- The pathogenic species are mostly intracellular.

Biochemical reactions:

- Oxidase test: +ve; all species produce oxidase enzyme.
- Sugar fermentation to differentiate pathogenic species from commensals:

Microorganism	Glucose	Maltose	Sucrose
<i>N. meningitidis</i>	+	+	-
<i>N. gonorrhoeae</i>	+	-	-
<i>Morexella catarrhalis</i>	-	-	-
<i>N. sicca</i>	+	+	+

### ***Neisseria meningitides***

#### Pathogenesis & Clinical Findings

Humans are the only natural hosts for whom meningococci are pathogenic. The nasopharynx is the portal of entry. There, the

organisms attach to epithelial cells with the aid of pili; they may form part of the transient flora without producing symptoms. From the nasopharynx, organisms may reach the bloodstream, producing bacteremia; the symptoms may be like those of an upper respiratory tract infection.

Fulminant meningococemia occurs due to multiplication of bacteria in the blood stream, it is more severe, with high fever and hemorrhagic petechiae (rash); there may be disseminated intravascular coagulation and circulatory collapse (Waterhouse-Friderichsen syndrome). The highest levels of endotoxin measured in sepsis have been found in patients with meningococemia (50- to 100-fold greater than with other gram-negative infections).

In meningitis, the meninges are acutely inflamed, with thrombosis of blood vessels and exudation of polymorphonuclear leukocytes, so that the surface of the brain is covered with a thick purulent exudate.

It is not known what transforms an asymptomatic infection of the nasopharynx into meningococemia and meningitis, but this can be prevented by specific bactericidal serum antibodies against the infecting serotype. *Neisseria* bacteremia is favored by the absence of bactericidal antibody (IgM and IgG), inhibition of serum bactericidal action by a blocking IgA antibody, or a complement component deficiency.

## Virulence Factors

### Capsules

Presence of capsules provide resistance against antibody, complement-mediated, or phagocytic destruction by the host immune response.

### LPS

Endotoxin is a cardinal virulence factor, inducing septic shock in patients by triggering pro-inflammatory mediator production.

### Adhesion

Several structural and molecular features allow bacteria to adhere to the mucosal surfaces for colonization. *N. meningitidis* use pili located on its surface and proteins mediate adhesion and invasion of host cells to facilitate the spread of the pathogen.

### **Diagnostic tests:**

Meningococcus samples: blood, CSF.

1. Smear: Gram-stained smears.
2. Culture: Specimens are streaked on chocolate agar & on selective medium e.g. modified Thayer-Martin. Medium incubated in 5% CO<sub>2</sub> environment at 37°C.
3. Nasopharyngeal swab cultures are suitable for carrier surveys.
4. Puncture material from petechiae may be taken for smear and culture.
5. Serology: Antibodies to meningococcal polysaccharides can be measured by latex agglutination.

## **Prevention, & Control**

A rise in the number of cases is preceded by an increased number of respiratory carriers. Treatment with oral penicillin does not eradicate the carrier state. Chemoprophylaxis for household and other close contacts using rifampin or ciprofloxacin, can often eradicate the carrier state.

More important is the reduction of personal contacts in a population with a high carrier rate. This is accomplished by avoidance of crowding or administration of vaccines which contain the capsular polysaccharides as antigens.

## **Treatment**

Due to the severity of illness, any individual suspected of infection by *N. meningitidis* should be admitted to a hospital immediately. Penicillin G is the drug of choice for treating meningococcal disease. Either chloramphenicol or a third-generation cephalosporin such as cefotaxime or ceftriaxone is used in persons allergic to penicillins.

## ***Neisseria gonorrhoeae* (gonococcus GC)**

*N. gonorrhoeae* is a relatively fragile organism, susceptible to temperature changes, drying, UV light, and other environmental stresses. Strains of *N. gonorrhoeae* are fastidious and variable in their cultural requirements, so that media containing hemoglobin, yeast extract and other supplements are needed for isolation and growth of



the organism. Cultures are grown at 35-36°C in an atmosphere of 3-10% added CO<sub>2</sub>.

### **Pathogenesis & Clinical Findings**

The infections are acquired by sexual contact (or direct contact, in the case of infections in the newborn). The pathogen invades the spaces separating columnar epithelial cells (of both male and female), which are found in the oral-pharyngeal area, the eyes, rectum, urethra and opening of the cervix causing infections such as pharyngitis, conjunctivitis, urethritis, cervicitis and pelvic inflammatory diseases. The invasion sets up an inflammation and, when leukocytes move into the inflamed area, the characteristic pus forms.

Males become aware of a gonorrheal infection by painful urination and a discharge of pus-containing material from the urethra. In females, the primary infection is in the endocervix and extends to the urethra and vagina, giving rise to mucopurulent discharge. If the mother is infected with gonorrhea, the eyes of the infant can become infected as it passes through the birth canal and can result in blindness.

Gonococcal bacteremia leads to skin lesions and the untreated gonorrhea can be systemic infection. Complications can involve the heart (*gonorrheal endocarditis*), meninges (*gonorrheal meningitis*), or joints *Gonorrheal arthritis*, also eyes and pharynx,

Neisserial LPS is distinguished from enteric LPS by its highly oligosaccharide structure and the absence of repeating O-antigen subunits. For these reasons, neisserial LPS is referred to

as lipooligosaccharide (LOS) which triggers an intense inflammatory response.

Both *N. gonorrhoeae* and *N. meningitidis* produce IgA proteases which promote virulence. The organism is frequently found intracellularly in polymorphonuclear leukocytes (neutrophils) of the gonorrhea pustule exudate.

There is no effective adaptive immunity to gonorrhea. The conventional explanation is that the gonococcus exhibits extraordinary antigenic variability.

### **Virulence Factors:**

**A. Cell surface components:** Pili and Outer Membrane Proteins (Por, Opa, LOS,....).

-The porin of *N. gonorrhoeae* (Por) apparently has a role in virulence that allows the gonococci to survive inside of phagocytes.

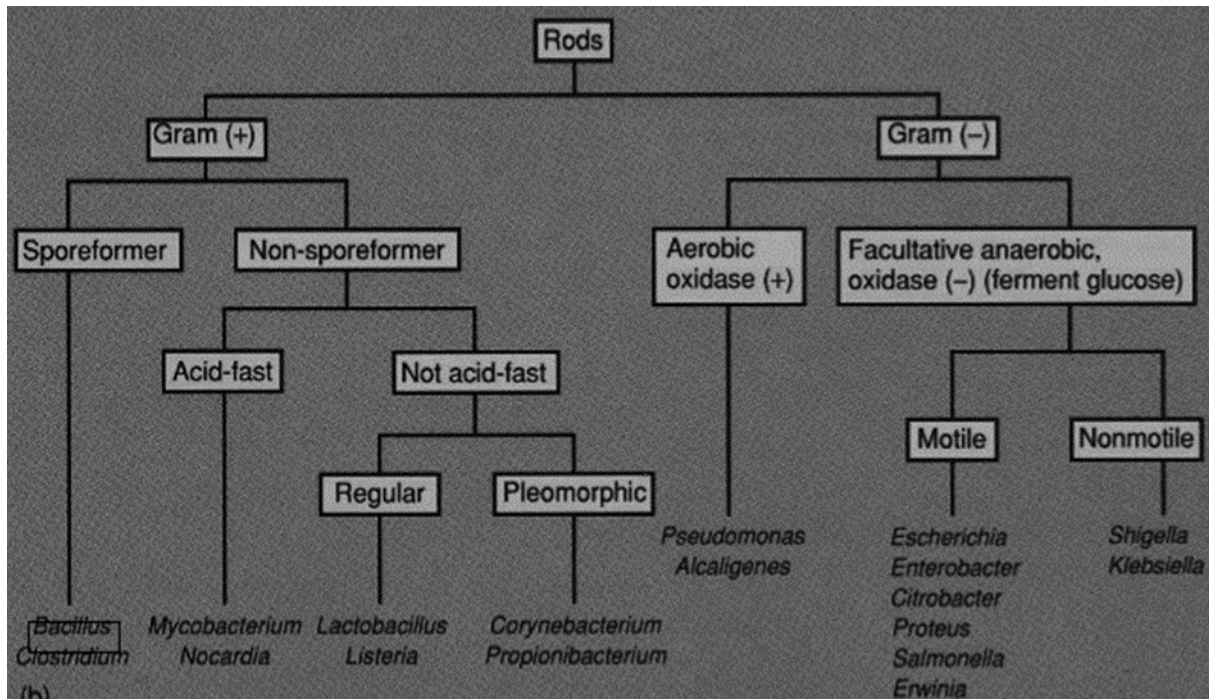
-Opa proteins function in adhesion of gonococci within colonies and in attachment of gonococci to host cell receptors.

**B. Extracellular Products:** Secretory IgA 1 protease

**Diagnosis of Gonorrhea:** Smear, Culture (selective; in Thayer-Martin and nonselective; in chocolate agar), ELISA, and Nucleic acid amplification tests.

### **Treatment**

Ceftriaxone, cefixime or Azithromycin are widely used; quinolones are no more recommended because of the rapid development of resistance.



The genus *Bacillus* includes large aerobic, gram-positive rods occurring in chains. Most members of this genus are saprophytic organisms prevalent in soil, water, and air and on vegetation, such as *Bacillus cereus* and *Bacillus subtilis*. *B anthracis* is a major agent of bioterrorism and biologic warfare, which causes anthrax. *Bacillus cereus* causes food poisoning and occasionally eye or other localized infections.

### Morphology & Identification

The typical cells, have square ends and are arranged in long chains; spores are located in the center of the nonmotile bacilli. Hemolysis is uncommon with *B anthracis* but common with *B cereus* and the saprophytic bacilli.

### *Bacillus anthracis*

**Pathogenesis:** Anthrax is primarily a disease of herbivores—sheep, cattle, horses, etc; other animals (eg, rats) are relatively resistant to the infection.

Humans become infected incidentally by contact with infected animals or their products. In animals, the portal of entry is the mouth and the gastrointestinal tract. In humans, the infection is usually acquired by the entry of spores through injured skin (cutaneous anthrax) or rarely the mucous membranes (gastrointestinal anthrax), or by inhalation of spores into the lung (inhalation anthrax). In humans, approximately 95% of cases are cutaneous anthrax.

The spores germinate in the tissue, a pruritic papule develops 1–7 days after entry and growth of the vegetative organisms results in formation of a gelatinous edema and congestion. The lesions typically are 1–3 cm in diameter and have a characteristic central black eschar. Bacilli spread via lymphatics to the bloodstream, and systemic signs and symptoms of fever, malaise, and headache may occur. After 7–10 days the eschar is fully developed. Eventually it dries, healing and leaves a scar. Antibiotic therapy does not appear to change the natural progression of the disease. In as many as 20% of patients, cutaneous anthrax can lead to sepsis, the consequences of systemic infection—including meningitis—and death.

*B anthracis* that does not produce a capsule is not virulent and does not induce anthrax in test animals. The capsule gene is on a plasmid.

Anthrax toxin is made up of three proteins: protective antigen (PA), edema factor (EF), and lethal factor (LF). The anthrax toxin genes are on another plasmid.

In inhalation anthrax (wool sorter disease), the spores are inhaled, phagocytosed in the lungs, and transported by the lymphatic drainage to the mediastinal lymph nodes, where germination occurs. This is followed by toxin production and the development of hemorrhagic mediastinitis and sepsis, which are usually rapidly fatal. In anthrax sepsis, the number of organisms in the blood exceeds  $10^7$ /mL just prior to death.

## **Diagnostic Laboratory Tests**

Specimens are fluid or pus from a local lesion, blood, and sputum.

-Stained smears from the local lesion or of blood from dead animals often show chains of large gram-positive rods.

-When grown on blood agar plates, the organisms produce nonhemolytic gray to white colonies.

-In semisolid medium, anthrax bacilli are always nonmotile, whereas related organisms (eg, *B cereus*) exhibit motility by "swarming."

-Gelatin hydrolysis is negative while other *Bacillus* species are positive.

-Lysis by a specific anthrax,  $\gamma$ -bacteriophage may be helpful in identifying the organism.

-An enzyme-linked immunoassay (ELISA) and may also nucleic acid amplification assays.

## **Treatment**

Many antibiotics are effective against anthrax in humans, but treatment must be started early. Ciprofloxacin is recommended for treatment; penicillin G, along with gentamicin or streptomycin, has previously been used to treat anthrax.

## ***Bacillus cereus***

Food poisoning caused by *B cereus* has two distinct forms: the emetic type, and the diarrheal type. *B cereus* produces toxins that cause disease that is more an intoxication than a food-borne infection.

The emetic form is manifested by nausea, vomiting, abdominal cramps, and occasionally diarrhea and is self-limiting, with recovery occurring within 24 hours. It begins 1–5 hours after ingestion of rice. *B cereus* is a soil organism that commonly contaminates rice. When large amounts of rice are cooked and allowed to cool slowly, the *B cereus* spores germinate, and the vegetative cells produce the toxin during log-phase growth or during sporulation. The enterotoxin is heat-stable.

The diarrheal form has an incubation period of 1–24 hours after ingestion of meat dishes and vegetables and is manifested by profuse diarrhea with abdominal pain and cramps. The enterotoxin is heat-labile.

**Diagnosis (For food poisoning):** The presence of *B cereus* in a patient's stool is not sufficient to make a diagnosis of *B cereus* disease, since the bacteria may be present in normal stool specimens (NO CAPSULE); a concentration of  $10^5$  bacteria or more per gram of food is considered diagnostic.

*B.cereus* is an important cause of eye infections, severe keratitis and endophthalmitis. 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.

**Treatment:** *B cereus* is resistant to a variety of antimicrobial agents including penicillins and cephalosporins. It is sensitive to vancomycin, chloramphenicol, ciprofloxacin, and gentamicin. Clindamycin with gentamicin, given early, is the best treatment for ophthalmic infections.

Other *Bacillus* species are rarely associated with human disease. There are five *Bacillus* species are pathogens for insects, and some have been used as commercial insecticides.

<b>Characteristics</b>	<b><i>B. anthracis</i></b>	<b><i>B.cereus</i></b>
<b>Hemolysis on BAP</b>	=	+
<b>Motility</b>	=	+
<b>Gelatin hydrolysis</b>	=	+
<b>Susceptibility to Penicillin (10U/ml)</b>	Susceptible	Resistant