Medical Microbiology

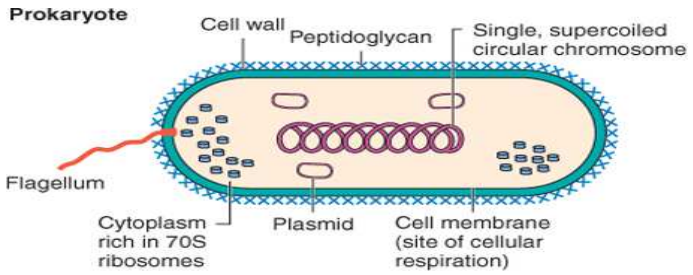
2nd and 3rd year of General Medicine

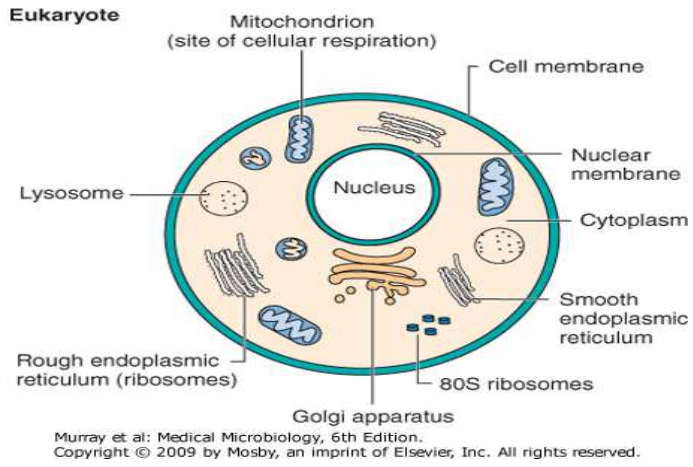
By Christos Dermitzakis

General Microbiology

1. Structure of bacterial cell

Bacteria, the smallest cells, are visible only with the aid of a microscope. Their size can vary between 0,1 μm (Rickettsia, Chlamydia) to many microns in length. Most, however, are in the range of 1μm in diameter.
The cytoplasm of a bacterial cell contains the DNA chromosome, mRNA, ribosomes, proteins and metabolites. The bacterial chromosome is a single, double-stranded circle that isn't contained in the nucleus but a structure called the nucleoid. Histones aren't present. Plasmids, which are much smaller, circular, extrachromosomalDNAs, may also be present.
The lack of a nuclear membrane simplifies the requirements and control mechanisms for the synthesis of proteins.
The bacterial ribosome consists of 30S and 50S subunits, forming a 70S ribosome. The bacterial proteins are significantly different from those of eukaryotic cells.
The cytoplasmic membrane has a lipid bilayer structure similar to that of eukaryots but contains no steroids (exception, mycoplasma). This membrane is important for many functions such as electron transport and energy production. The inside of the cell is lined by actin-like proteins that determine the shape of the bacteria.
The structure, components and functions of the cell wall distinguish gram-positive from gram-negative bacteria. Cell wall components are also unique to bacteria and their repetitive structures elicit innate protective immune responses in humans.
The important differences are the peptidoglycan layers that surround the cytoplasmic membranes (exception, achaeobacteria, mycoplasma). Because the peptidoglycan provides ridgidity, it also helps to determine the shape of the particular bacterial cell. Gram-negative bacteria are also surrounded by outer membranes.





2. Microscopic diagnostics of microorganisms. Diagnostic staining methods (Gram, Ziehl-Neelsen)

Diagnostics of illnesses of microbial etiology can be concentrated on primary identification of the causative agent. Infectious diseases can be diagnosed on the basis of indirect methods.

Direct diagnostic methods include:

a) macroscopic evaluation – the sample is visualized by the naked eye, e.g. presence of a worm in stool, blood and mucous in sputum etc
b) microscopic examination – the light microscope is used for most bacteria, fungi and parasites but rarely for viruses (they're too small). Immunofluoresence can be used to detect intracellular bacteria and viruses. Viruses are generally detected by electron microscopes
c) detection of antigens in the material (bacterial capsules, toxins, viral antigens)
d) detection of a specific microbial nucleic acid in the material
e) cultivation/isolation is carried out commonly in detecting bacteria and fungi, in specific culture media and under specific environmental conditions
f) identification of the causative agent following culture – according to microscopy, biochemical tests, sensitivity to bacteriophages, antigens, specific nucleic acids etc
g) detection of susceptibility towards anti-infectious therapeutics of identified causative agents

Indirect diagnostic methods can be divided into serological methods and skin tests. Many serological methods are basically used in the detection of antibodies against antigens of viruses, bacteria, parasites in a lesser use and only rarely in the case of fungi. Formation of IgM antibodies indicates mostly an ongoing disease. In some diseases, the diagnosis is aimed towards the detection of cell-mediated reactions by skin tests. Even though clinicians don't usually perform these tests, it must be mentioned that skin tests are specifically important in the diagnostics of certain diseases such as tuberculosis.

Gram staining belongs to the group of basic diagnostic stains in clinical microbiology. According to the results, bacteria are divided into gram-positive and gram-negative. The difference is in the cell wall of the microorganisms.

The procedure includes:

a) add the primary stain crystal violet to the specimen on the slide and wait 3 minutes
b) add gram's iodine solution for 30 seconds or Lugol's solution for 2 minutes
c) wash with ethanol and acetone, the decolourisers, for 5 seconds
d) rinse gently with water
e) add the secondary stain carbolfuchsin or safranin for 1 minute
f) wash with water for a maximum of 5 seconds
g) observe under 1000x magnification with the aid of immersion oil

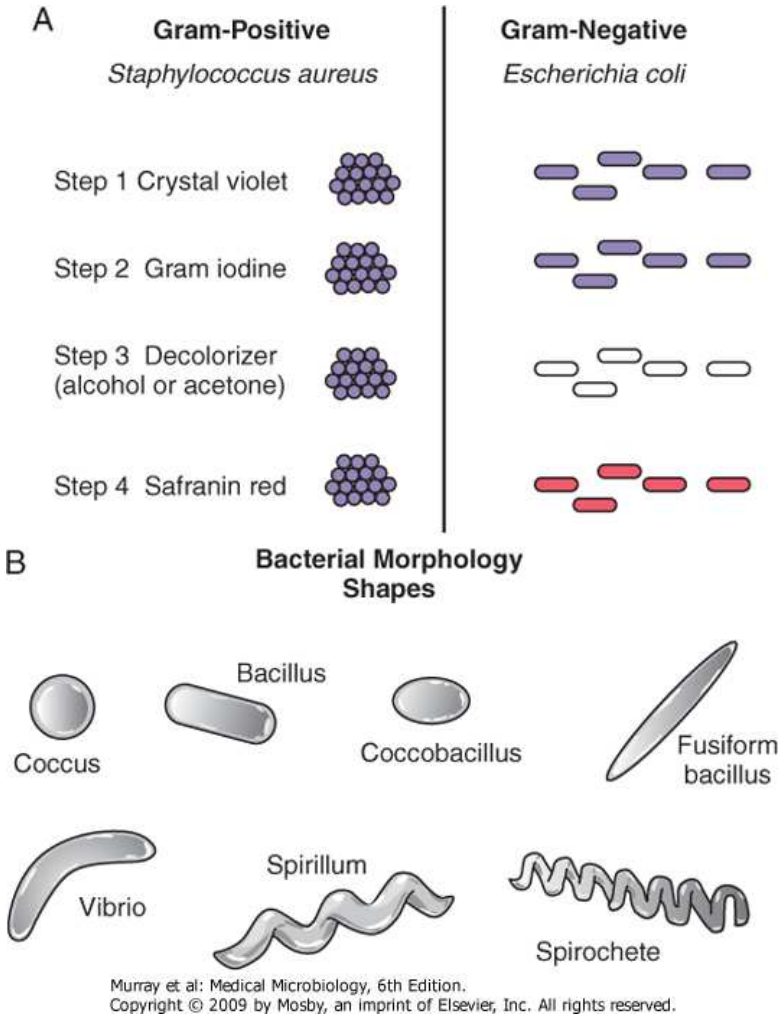
The result is visualization of blue-violet gram-positive bacteria and red gram-negative bacteria.

Acid-fast bacteria are characterized by their extraordinary resistance against the impact of chemical agents, as opposed to other bacteria, which are hardly decolourised by acids and non-polar solvents. Ziehl-Neelsen staining uses the high retention of carbolfuchsin following decolourisation of acid-fast bacteria by acidic alcohol. Before visualization, acid-fast bacteria are stained by a counter-stain.

The procedure includes:

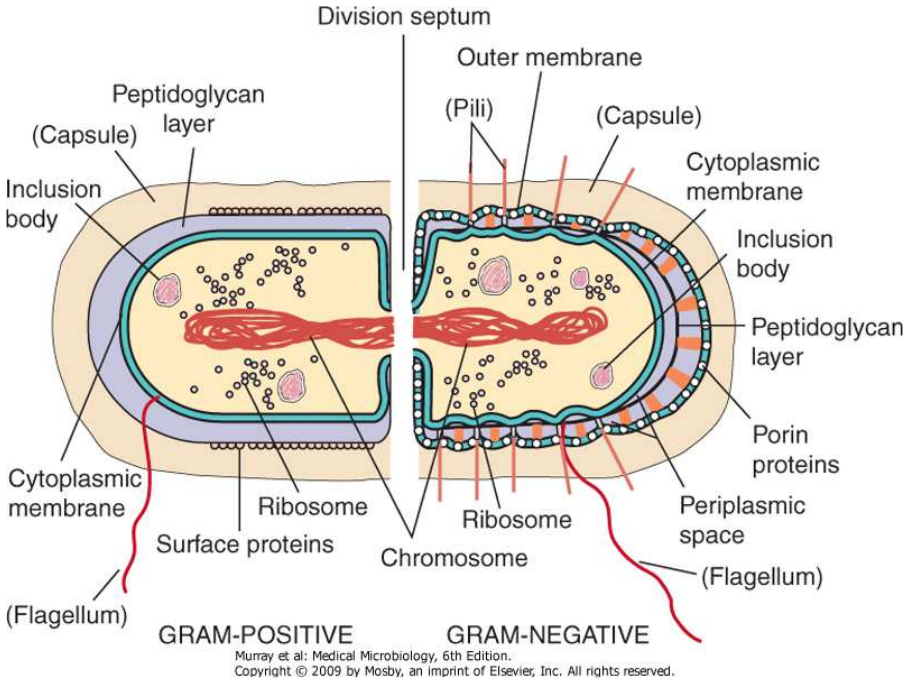
a) concentratedcarbolfuchsin is poured over a fixed preparation and heated over a flame until vapor arises (repeated 3 times)
b) wash with water gently for 1 minute
c) recolourise with methylene blue
d) wash with water for 1 minute and leave to dry in the air
e) observe under 1000x magnification with the aid of immersion oil

In the smear are seen red-stained rods which usually form aggregates. Then the acid-fast rod count is carried out according to their number (for 0, -, for up to 20, +, for 21-100, ++, for more than 100, +++)



3. Structure of cell wall in gram-positive, gram-negative and mycobacteria and its role in pathogenesis

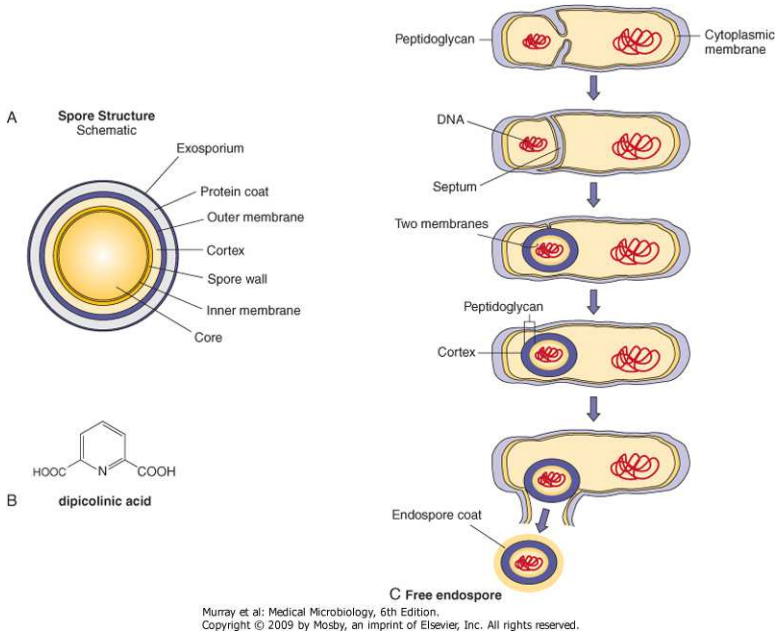
A gram-positive bacterium has a thick, multilayered cell wall consisting mainly of peptidoglycan, surrounding the cytoplasmic membrane. The peptidoglycan is a mesh-like exoskeleton similar in function to the exoskeleton on an insect. This peptidoglycan is significantly porous to allow diffusion of metabolites to the plasma membrane. The peptidoglycan is essential for the structure, replication and survival in the normally hostile conditions in which bacteria grow.
The peptidoglycan can be degraded by treatment with lysozyme.
The gram-positive cell wall may also include other components such as teichoic acid and lipoteichoic acid and complex polysaccharides. Teichoic acids are water-soluble, anionic polymers of polyol phosphates, which are covalently linked to the peptidoglycan. Lipoteichoic acids have a fatty acid and are anchored in the cytoplasmic membrane. These molecules are common surface antigens that distinguish bacterial serotypes and promote attachment to other bacteria. Teichoic acids are an important factor of virulence. Lipoteichoic acids are shed into the media and the host and although weaker, they can initiate innate protective responses similar to endotoxin.



Gram-negative cell walls are more complex, both structurally and chemically. Structurally, a gram-negative cell wall contains two layers external to the cytoplasmic membrane. Immediately external to it is a thin peptidoglycan layer which accounts only for 5-10% of the cell wall by weight. There are no teichoic or lipotheichoic acids. External to the peptidoglycan layer is the outer membrane, which is unique to gram-negative bacteria. The area between the cytoplasmic membrane and the outer membrane is called the periplasmic space. Here usually reside transport systems for iron, proteins. The outer membrane is like a stiff canvas sack around the bacteria. It maintains the bacterial structure and is a permeability barrier to large molecules and hydrophobic molecules. It also provides protection against adverse environmental conditions.

The outer membrane has an asymmetric bilayer structure that differs from any other biologic membrane in the structure of the outer leaflet. The inner leaflet is composed primarily of lipopolysaccharide (LPS). LPS is also called endotoxin, a powerful stimulator of innate and immune responces. LPS is shed from the bacteria into the media and host. LPS activates B cells and induces macrophages, dendritic cells and other cells to release IL-1, IL-6 and TNF. LPS induces fever and can cause shock.
The variety of proteins found in gram-negative outer membranes is limited, but several of the proteins are present in high concentration, resulting in a total protein content that is higher than that of the cytoplasmic membrane. A group of proteins called porins form pores that allow diffusion of hydrophilic molecules through the membrane.
The outer membrane is connected to the cytoplasmic membrane at adhesion sites and is tied to the peptidoglycan by lipoprotein.
Mycobacteria have a peptidoglycan layer which is intertwined with and covalently attached to an arabinogalactan polymer and surrounded by a waxlike lipid coat of mycolic acid, cord factor, wax D and sulfolipids. These bacteria are described as acid-fast staining. The coat is responsible for virulence and is antiphagocytic.
Corynebacterium and Nocardia organisms also produce mycolic acid lipids. The mycoplasms are also exceptions in that they have no peptidoglycan.

4. Bacterial spores, their morphology and biological role



Some gram-positive, but never gram-negative, bacteria, such as members of the Bacillus and Clostridium families, are spore formers. Under harsh environmental conditions, these bacteria can convert from a vegetative state to a dormant state or spore. The location of the spore within the cell is characteristic of the bacteria and helps with their identification.
The spore is a dehydrated, multishelled structure that protects and allows the bacteria to exist in “suspended animation”. It contains a complete copy of the chromosome and a high concentration of calcium bound to dipicolinic acid.

The spore has an inner membrane, two peptidoglycan layers and an outer keratin-like protein coat. This structure of the spore protects the genomic DNA from intense heat, radiation and attack from most enzymes and chemical agents.
Depletion of specific nutrients (alanine) from the growth medium triggers a cascade of genetic events leading to the production of the spore. Spore mRNA are transcribed and other mRNA are turned off. Dipicolinic acid is produced and antibiotics and toxins are often excreted. After duplication of the chromosome, one copy of the DNA and cytoplasmic contents (core) are surrounded by the cytoplasmic membrane, the peptidoglycan and the membrane of the septum. The two peptidoglycan layers are surrounded by the cortex. The cortex is surrounded by the tough, keratin-like protein coat. The process requires 6 to 8 hours for completion.
The germination of spores into the vegetative state is stimulated by disruption of the outer coat by mechanical stress, pH, heat or another stressor and requires water and a triggering nutrient (alanine). The process takes about 90 minutes. After the germination of spores begins, the spore will take up water, swell, shed its coats and produce one new vegetative identical cell, thus completing the entire cycle. Once germination has begun and the coat has been compromised, the spore is weakened, vulnerable and can be inactivated like other bacteria.

5. Growth, multiplication and metabolism of microorganisms, growth curve. Role in classification and identification

Bacterial growth requires a source of energy and the raw materials to build the proteins, structures and membranes that make up and power the cell.
The minimum requirement for growth is a source of carbon and nitrogen, an energy source, water and various ions. Essential elements include components of proteins, lipids and nucleic acids (C, O, H, N, S, P), important ions (Na, K, Mg, Ca, Cl) and components of enzymes (Fe, Zn, Mn, Mo, Se, Co, Cu, Ni).
Iron is so important that many bacteria secrete special proteins (siderophores) to concentrate iron from dilute solutions.
Oxygen, although essential for the human host, is actually a poison for many bacteria. Some organisms, such as Clostridium perfingens, which causes gas gangrene, can’t grow in the presence of oxygen. Such bacteria are referred to as obligate anaerobes. Other organisms such as Mycobacterium tuberculosis, which causes tuberculosis, require presence of molecular oxygen and are therefore termed as obligate aerobes. Most bacteria, however, grow in either presence of absence of oxygen. Such bacteria are called facultative anaerobes.
All cells require a constant supply of energy to survive. This energy, typically in the form of ATP, is derived from the controlled breakdown of carbohydrates, lipids and proteins. This process is called catabolism. The energy produced may be used for the synthesis of cellular components etc, a process known as anabolism. Together these processes are called intermediary metabolism.
The metabolic process generally begins with the hydrolysis of biologic macromolecules by specific enzymes. The metabolites are converted via one or more pathways to one common, universal intermediate, pyruvic acid.

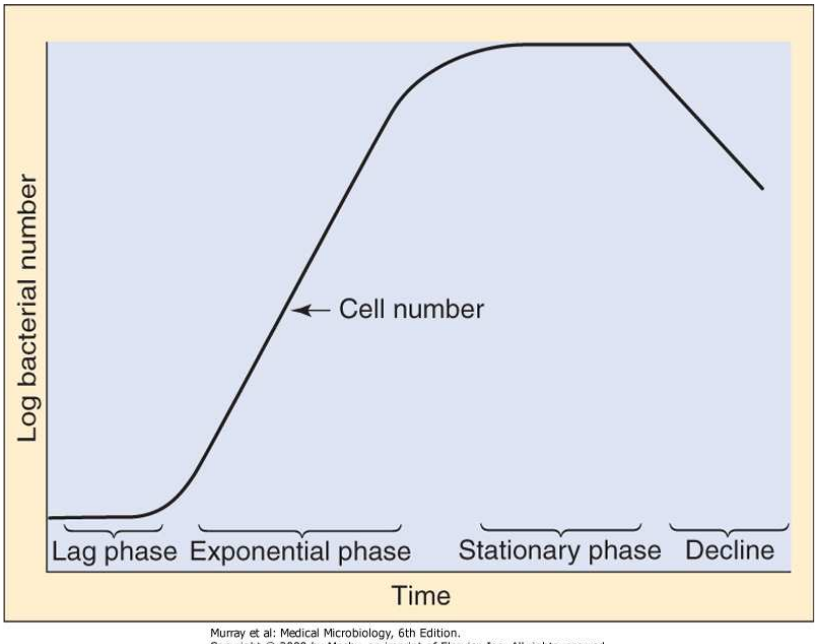
Carbohydrates are broken down to glucose, which enters a process called glycolysis, at the end of which glucose is turned into CO_2 and H_2O while in the process ATP is formed by various ways. The pyruvic acid produced enters the Krebs cycle where it gives off large amounts of ATP under aerobic conditions. This doesn't take place under anaerobic conditions.

Bacterial replication is a coordinated process in which two equivalent daughter cells are produced. For growth to occur, there must be sufficient metabolites to support the synthesis of the bacterial components. A cascade of regulatory events must occur on schedule to initiate a replication cycle. However, once it's initiated, DNA synthesis must run to completion, even if all nutrients have been removed from the medium.

Chromosome replication is initiated at the membrane, and each daughter chromosome is anchored to a different part of the membrane. As the bacterial membrane grows, the daughter chromosomes are pulled apart. Chromosome replication also initiates the process of cell division.

Depletion of metabolites or a buildup of toxic byproducts triggers the production of chemical alarmones, which causes synthesis to stop, but degradative processes to continue. Septum formation may be initiated but cell division may not occur. Many cells die. Similar signals may stimulate sporulation in species capable of this process.

When bacteria are added to a medium, they require time to adapt to the new environment before they begin dividing. This hiatus is known as the lag phase of growth. During the log or exponential phase, the bacteria will grow and divide with a doubling time characteristic of the strain and determined by the conditions. The number of bacteria will increase to 2^n, in which n is the number of generations. The culture eventually runs out of metabolites, or a toxic substance builds up in the medium. The bacteria then stop growing and enter the stationary phase.



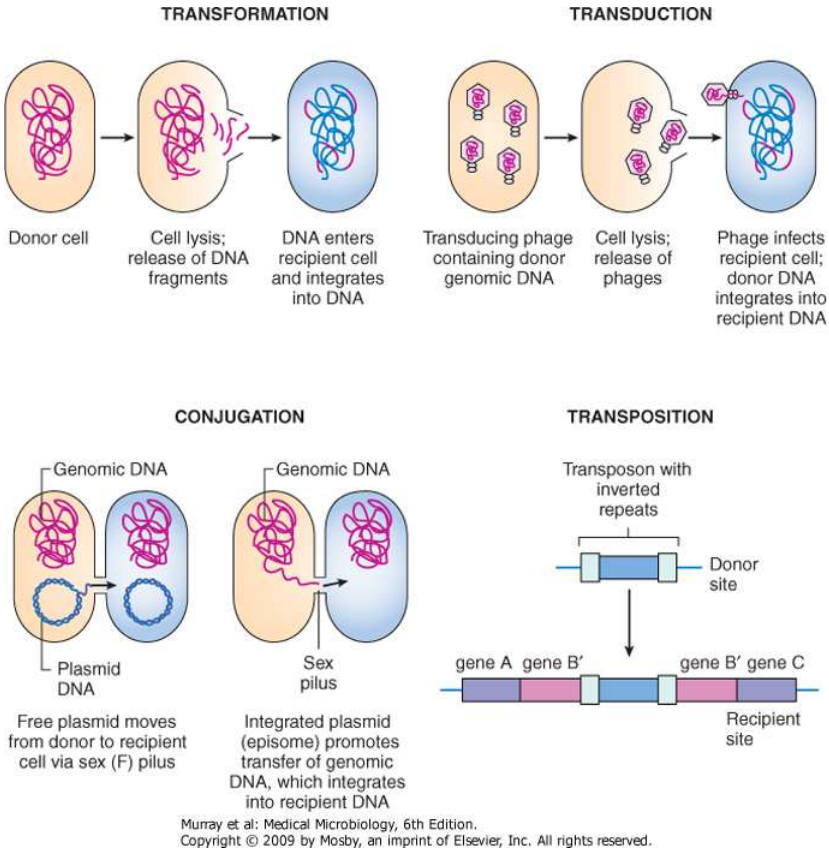
6. Conditions for cultivation of medically important bacteria

Cultivation is a procedure that ensures growth and multiplication of microorganisms under laboratory conditions. The aim of cultivation is microbiological diagnostics is to capture, isolate and multiply the investigated microorganism.
Processing and inoculation of the specimen should be performed under sterile conditions and proper processing must be secured. It's necessary to choose a cultivation medium, which suits the nutritional requirements of the tested microorganism and contains the required growth factors, vitamins etc. It's also necessary to secure an optimal pH (for most pathogenic bacteria it's 7.2-7.5). Humidity must be achieved in the media relevant for the diffusion of nutrients and metabolic processes. Optimal temperature for most human pathogens is 37^{o}C. Most medically important bacteria are usually cultured for 24-48 hours with exceptions of anaerobic bacteria, which may require more than 2 days and Mycobacterium tuberculosis, which requires 3-12 weeks. Partial pressure of oxygen and carbon dioxide play a major role in stabilizing the gaseous environment. Microorganisms, which require carbon dioxide for their multiplication are classified as capnophilic.

7. Phenotype and genotype changes (mutations, gene transfer)

A mutation is any change in the base sequence of the DNA. A signel base change can result in a transition in which one purine is replaced by another purine, or in which a pyrimidine is replaced by another pyrimidine. A transversion is the replacement of a purine with a pyrimidine. A silent mutation is a change at the DNA level that has no result on the phenotype. A missense mutation results in a different amino acid being inserted into the protein, which may be a conservative mutation if the new amino acid has similar properties. A nonsense mutation changes a codon encoding an amino acid to a stop codon. Conditional mutations such as temperature-sensitive mutations may result from conservative mutations, which change the structure of the protein due to temperature change.
More drastic changes can occur when numerous bases are involved. A small deletion that is not multiples of three produces a frameshift mutation. This results in a change in the reading frame, usually leading to wrong encoding of proteins. Null mutations, which completely destroy gene function, arise when there is an extensive insertion, deletion or gross rearrangement of the chromosome structure.
Many mutations happen spontaneously in nature, however physical or chemical agents can induce them. Physical agents inducing mutations in bacteria include heat, ultraviolet light, x-rays and others. Chemical agents can be grouped into nucleotide-base analogs, that cause mispairing of DNA, frameshift mutagens, which increase the spacing of specific base pairs and DNA-reactive chemicals, which act directly on the DNA to change the chemical structure.
The exchange of genetic information between bacterial cells may occur by one of three mechanisms, conjugation, transformation or transduction.
Transformation if the process by which bacteria take up fragments of naked DNA and incorporate them into their own genomes. Gram-positive and gram-negative bacteria can take up stably maintain exogenous DNA. Certain species are naturally capable of this property including Haemophilusinfluenzae, Streptococcus pneumoniae, Bacillus species

and Neisseria species. Most bacteria don’t exhibit a natural ability for DNA uptake. Chemical methods can be used to introduce plasmids and other DNA into E. coli and other bacteria.



Conjugation occurs with most, if not all, eubacteria. It usually occurs between members of the same or related species, but has also been demonstrated between prokaryotes and cells from plants, animals and fungi. Conjugation occurs for E. coli, bacteroides, enterococci, streptococci, streptomyces and clostridia.
Conjugation results in the one-way transfer of DNA from a donor cell to a recipient cell through the sex pilus. The mating type (sex) of the cell depends on the presence or absence of a conjugative plasmid, such as the F plasmid of the E. coli.
Transduction is the genetic transfer mediated by bacterial viruses (bacteriophages), which pick up fragments of DNA and package them itno bacteriophage particles. The DNA is delivered to infected cells and becomes incorporated into the bacterial genomes. Transduction can be classified as specialised if the phages transfer particular genes or generilised if the selection of the sequence is random because of accidental packaging of host DNA into the phage capsid.

8. Antigens and antigenic structure of microorganisms, role in classification and identification

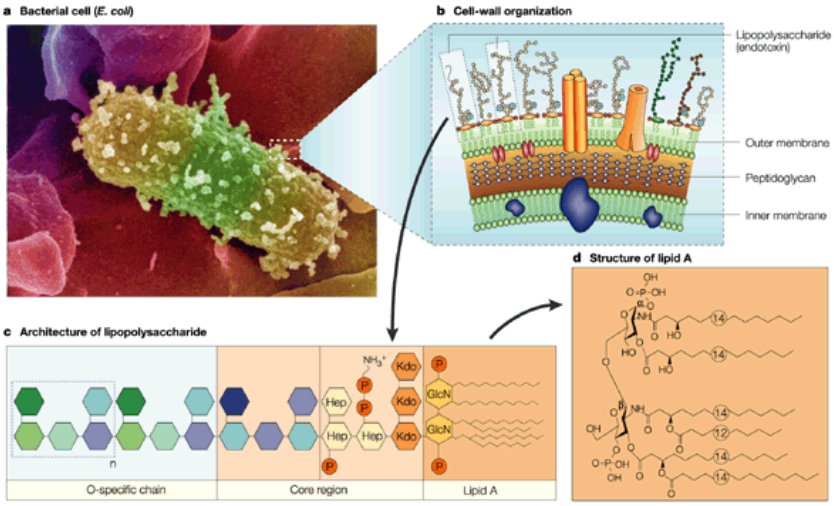
Almost all of the proteins and carbohydrates associated with an infectious agent are considered foreign to the human host and have the potential to initiate an immune response.
An antigen is a molecule that is recognised by specific antibody or T cells. An epitope (antigenic determinant) is the actual molecular structure that interacts with a single antibody molecule or T cell receptor.

9. Pathogenesis, virulence and its changes

Pathogenecity is the ability of a microorganism to cause a disease in a specific host. Pathogens that infect humans are called anthropopathogenic, those that infect plants are called phytopathogenic and those that infect animals are called zoopathogenic. Pathogenesis is species specific.
Pathogens can be classified into primarily pathogenic (or strictly or obligatory) which are able to cause a disease in immunocompetent and non-immune hosts (e.g. Streptococcus pyogenes, Salmonella typhi) and secondarily pathogenic (or facultative) which are able to cause a disease only in immunocompromised hosts (e.g. Pseudomonas aeruginosa).
Virulence is the degree of pathogenecity, the degree of ability to damage the host and is mediated by virulence factors (spectrum and produced amounts). Virulence is strain specific. It's decreased with cultivation in non – appropriate environments or by genetic engineering (vaccines) or is increased with cultivation in a susceptible host (natural selection) or by genetic engineering (bioterrorism).
Virulence factors contribute to pathogenesis of a disease and include attachment, survival and multiplication (colonization), spreading in the host (infection) and damage to the host body structure and function (clinical disease). These factors are usually coded for on plasmids or phages.
Adhesins – mediate firm attachment to the surfaces in the host
Invasins – mediate invasion of the bacteria into the host cellsand tissue
Impedines – impede host defense mechanisms
Agressins – damage the host tissue or organ structure or function
Modulins – stimulate inflammatory cytokines release andinflammatory reactions

10. Endotoxin and its significance

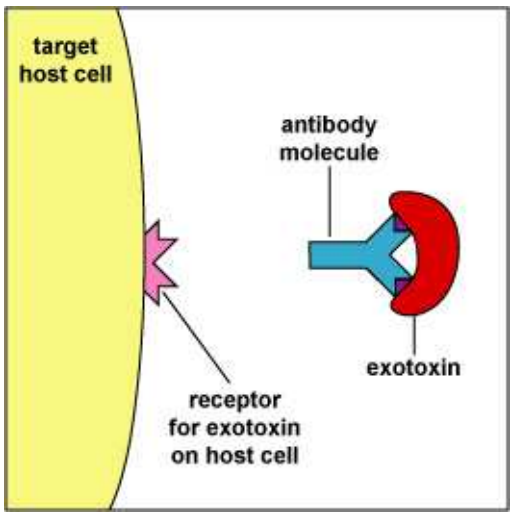
An endotoxin is a structural part of the bacterial cell wall of Gram-negative bacteria calledlipopolysaccharide. It induces a very powerful pyrogenic (fever) acute phase response and inflammatory reactions.



Lipopolysaccharide is released in appropriate amounts localized at the infectious site. In large amounts in the blood stream it's involved in the development of sepsis syndrome as a response to Gram-negative bacterial infection, which may lead to septic shock.
Disseminated intravascular coagulation can also result from the activation of blood coagulation pathways. The high fever, skin lesions and potential symptoms of shock associated with Neisseriameningitidis infection can be related to large amounts of endotoxin released during infection.
The lipid A portion of lipopolysaccharide is responsible for endotoxin activity. Endotoxin is different from exotoxin and only Gram-negative bacteria produce endotoxin.

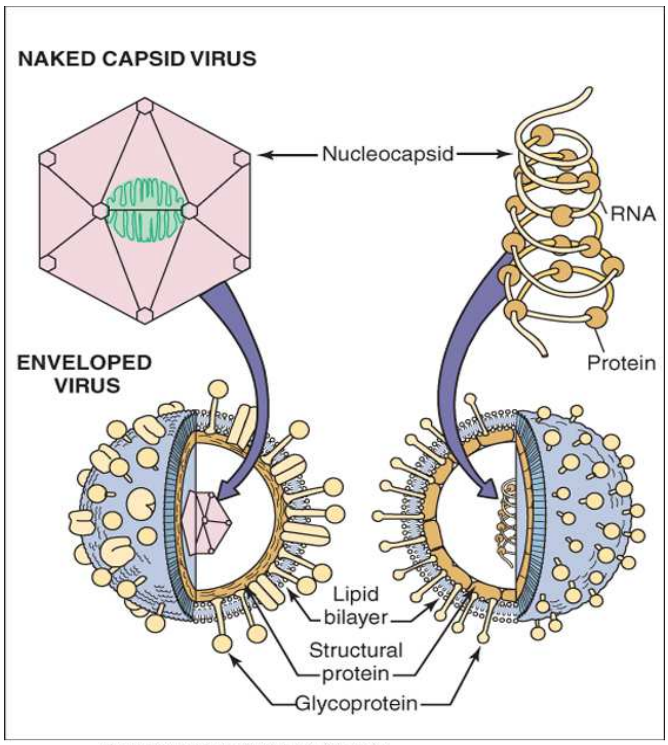
11. Bacterial exotoxins. Classficiation and role in pathogenesis and diagnosis

Exotoxins are proteins that can be produced by Gram-positive or Gram-negative bacteria and include cytolytic enzymes and receptor-binding proteins that alter a function or kill the cell. Usually, the toxin produced is a plasmid (e.g. tetanus toxin of Clostridium tetani).
Cytolytic toxins include membrane-disrupting enzymes such as the a-toxin (phospholipase C) produced by Clostridium perfringens, which breaks down the sphingomyelin and other membrane phospholipids. Haemolysins insert into and disrupt erythrocytes. Pore forming toxins, including streptolysin O, can promote leakage of ions and water from the cell and disrupt cell functions or cell lysis.
Superantigens are a special group of toxins. These molecules activate T cells by binding simultaneously to a T cell receptor and a major histocompatibility complex class II molecule on an antigen-presenting cell without requiring antigen. Superantigens activate large numbers of T cellsto release large amounts of interleukins (e.g. IL-1, IL-2, TNF) causing life-threatening autoimmune diseases. Superantigens include the toxic shock syndrome toxin of Streptococcus aureus, staphylococcal enterotoxins and the erythrogenic toxin A or C of Streptococcus pyogenes.



12. General characteristics of viruses

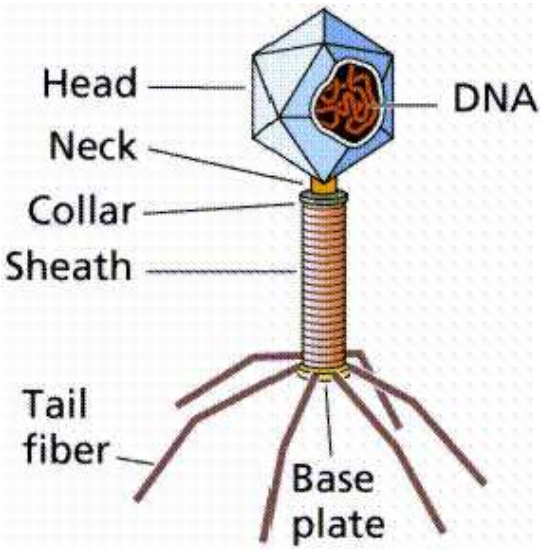
Viruses are obligate intracellular parasites that depend on the biochemical machinery of the host cell for replication. In addition, reproduction of viruses occurs by assembly of the individual components rather than by binary fission.
Viruses range from the structurally simple and small parvoviruses to the large and complex poxviruses and herpeviruses. They can be group by characteristics such as disease, target tissue, means of transmission or vector etc.
The units of measurement of virion size are nanometers. The clinically important viruses range from 18nm to 300nm.
The virion (viral particle) consists of a nucleic acid genome package into a protein coat (capsid) or a membrane (envelope). The virion may also contain certain essential or accessory enzymes or other proteins to facilitate replication in the cell. Capsid or nucleic acid-binding proteins may associate with the genome to form a nucleocapsid, which may be the same as the virion or surrounded envelope.
The genome of the virus can either be DNA or RNA. The DNA can be single stranded or double stranded, linear or circular. The RNA can be either positive sense or negative sense, double stranded or ambisense. The RNA genome may also be segmented into pieces, with each piece encoding one or more genes.



The outer layer of the virion is the capsid or envelope. These structures are the package, protection and delivery vehicle during transmission of the virus. The surface structures of the capsid and envelope mediate the interaction of the virus with the target cell through a viral attachment protein or structure. Removal or destruction of the outer package inactivates the virus.

The capsid is a rigid structure able to withstand harsh environmental conditions. Viruses with naked capsids are generally resistant to drying, acid and detergents. Many of these viruses are transmitted bt the fecal-oral route and can endure transmission even in sewage.
The envelope is a membrane composed of lipids, proteins and glycoproteins. The membranous structure of the envelope can be maintained only in aqueous solutions. It’s readily disrupted by drying, acidic conditions, detergents and solvents such as ether. As a result, enveloped viruses must remain wet and are generally transmitted in fluids, respiratory droplets, blood and tissues. Most can’t survive the harsh conditions of the gastrointestinal tract.

13. General characteristics of bacteriophages, their use in microbiological diagnostics



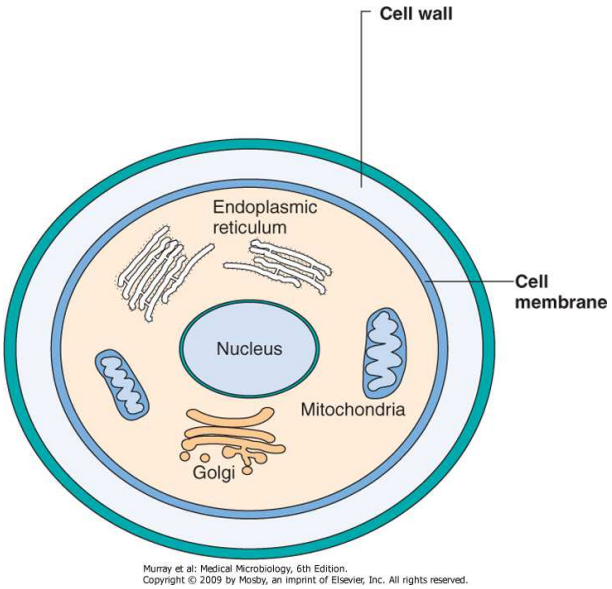
A bacteriophage is any one of a number of viruses that infect bacteria. Bacteriophages are among the most common biological entities on Earth.The term is commonly used in its shortened form, phage.Typically, bacteriophages consist of an outer protein capsid enclosing genetic material. The genetic material can be single-stranded DNA or RNA or double-stranded DNA or RNA along with either circular or linear arrangement.The double-stranded DNA tailed phages, or Caudovirales, account for 95% of all the phages. Generally, phages are classified according to their morphology and nucleic acid. The most characteristic examples of bacteriophages are T4 and λ (lambda). Bacteriophages have a lytic cycle or a lysogenic cycle and a few viruses are capable of carrying out both. With lytic phages such as the T4 phage, bacterial cells are broken open (lysed) and destroyed after immediate replication of the virion. As soon as the cell is destroyed, the phage progeny can find new hosts to infect.

In contrast, the lysogenic cycle doesn't result in immediate lysing of the host cell. The viral genome will integrate with host DNA and replicate along with it fairly harmlessly, or may even become established as a plasmid. The virus remains dormant until host conditions deteriorate and then the endogenous phages become active. At this point they initiate the reproductive cycle, resulting in lysis of the host cell. As the lysogenic cycle allows the host cell to continue to survive and reproduce, the virus is reproduced in all of the cell's offspring.

14. Morphology and physiology of medically important microscopic fungi

The fungi represent a ubiquitous and diverse group of organisms, the main purpose of which is to degrade organic matter. All fungi live a heterotrophic life a saprobes (organisms that live on dead or decaying matter), symbiots (organisms that live together

and the association is of mutual advantage), commenslas (organisms living in a close relationship) or as parasites (organisms that live on or within a host from which they derive benefits with no useful contribution). There are no nonpathogenic fungi.
Fungi are classified in their own separate kingdom. They are eukaryotic organisms that are distinguished from other eukaryotes by a rigid cell wall composed of chitin and glucan and a cell membrane in which ergosterol substitutes cholesterol.
Classic fungal taxonomy relies on morphology and mode of spore formation. According to their morphology, they can be divided into yeasts and molds. Yeasts reproduce by budding or fission, where a progenitor cell loses a portion of itself to produce a progeny of daughter cells. These cells then elongate to form pseudohyphae. Yeasts are usually unicellular. Molds on the other hand are multicellular organisms consisting of hyphae. Colonies formed by molds can be either filamentous, hairy or woolly. Many medically important fungi are termed dimorphic, meaning they can exist in both a yeast and mold form.



Most fungi exhibit aerobic respiration, although some are facultatively anaeroboic (fermentative) and others are strict anaerobs. Metabolically, fungi produce both primary (citric acid, ethanol, glycerol) and secondary (antibiotics, aflatoxins) metabolites. They are slow growing compared to bacteria.
In some fungi, the asexual stage of life has shown in contributing so greately to adaptation to new habitats, that the sexual stage has disappeared. Irrespective of the ability of a given fungus to produce sexual spores, it’s common to refer to the organisms by their asexual designations. Asexual spores consist of two general types, sporangiospores and conidia. The former are asexual spores produced in a containing structure or sporangia and ara characteristic of zygomycetes such as Rhizopus. Conidia are asexual spores that are borne naked on specialised structures as in Aspergillus, Penicillium and the dermatophytes.

15. General characteristics of agents causing human parasitosis

Parasitosis is the state in which a human suffers from a disease caused by a parasite. Human parasites are classified within the four eukaryotic kingdoms: protozoa, metazoa (animalia), fungi and chromista.
Protozoa are simple organisms that range in size from 2 to 100 μm. Their protoplasm is enclosed in a cell membrane. The nucleus contains clumped or dispressed chromatin and a central karyosome. They may possess flagella, cilia or pseudopods. Protozoa comprise 13 subgroups or phyla, of which 7 are of medical importance.
Flagellates, or mastigophora, move by lashing of their whip flagella. The number and position of the flagella vary in different species.
Amoebas, or amoebozoa, move by extursion of pseudopodia. They are phahgocytic and contain mitochondria.
Sporozoa, or coccidia, include a large group of sexually reproducing, spore-forming protozoans. These organisms have a system that produces substances to help it penetrate host cells and thus become an intracellular parasite.
Ciliates, or ciliophora, include a variety of free-living and symbiotic species. They move by coordinated movement of rows of hairlike structures similar to flagella. The only ciliated parasite of humans, Balantidium coli, contains two nuclei.
The chromista kingdom accommodates a number of plant-like organisms, like algae. The first one of these chromists known to parasitise humans is Blastocystis hominis.
Fungi, or else microsporidia, are small intracellular parasites that lack mitochondria. They are also characterised by the structure of their spores, which have a complex tubular extrusion used to inject the infective material into host cells.
Animalia, or metazoa, can be divided into two major groups, helminths (worms) and arthropods (insects, ticks, etc). Helminths are complex multicellular organisms that are elongated and bilateraly symmetrical. They range in size from less than 1mm to more than 1m. The protective covering of flatworms is know as the tegument. They often possess elaborate attachment structures such as hooks, teeth or plates. They are divided into two subgroups, nemathelminthes and platyhelminthes.
Arthropods are the largest group of animals in the kingdom of animalia. They are complex multicellular organisms that may be involved directly in causing invasive or superficial disease processes. In addition, envenomation by biting and stinging arthropods can result in adverse reactions such as allergic and hypersensitivity reactions or even anaphylactic shock and death. There are five major subgroups, myriapoda, pentastomida, crustecea, chelicerata and insecta.

16. Antimicrobial chemotherapeutics, classification according to the chemical structure, mechanisms and spectrum of activity

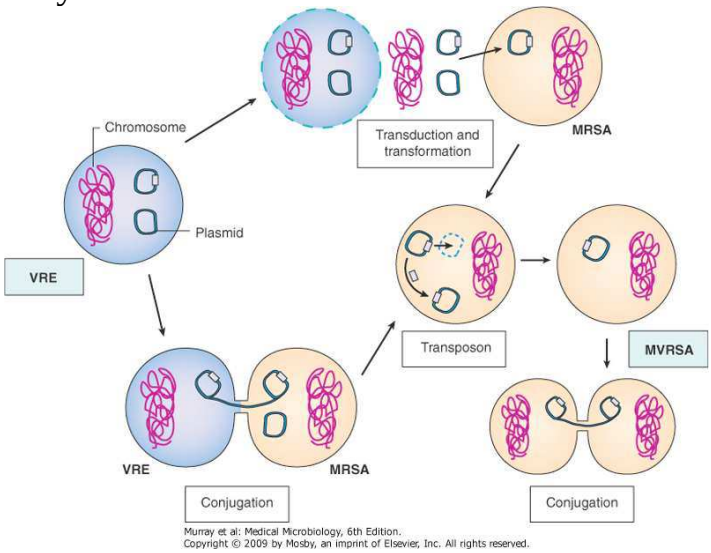
Antimicrobial chemotherapy is the use of specific antibiotics to treat a specific causative agent of a disease. Treatment can be either rational, according to the specific strain and its susceptibility to antiobiotics after microbiological testing, or empirical, which is in urget cases, according to the clinical signs of the disease.
Antimicrobial chemotherapeutics can be classified according to various critiria. One of them is chemical structure. To this division belong groups such as beta-lactams, glycopeptides, lipopeptides, polypeptides, aminoglycosides, quinolones, sulfonamides, glycylcyclines and others.

According to their mechanism of action, they can be divided into cell wall synthesis inhibitors (beta lactams, glycopeptides, lipopeptides, polypeptides, isoniazid, ethionamide, ethambutol, cycloserine) which inhibit synthesis of cell walls of bacteria. These are the most commonly used. Another group are protein synthesis inhibitors (aminoglycosides, tetracyclines, glycylcyclines, oxazolidinones, chloramphenicol, macrolides, ketolides, clindamycin, streptrogramins), which inhibit synthesis of proteins and are the second largest group. There are also nucleic acid synthesis inhibitors (quinolones, rifampin, metronidazole) which inhibit DNA or RNA synthesis. A last major group is that of antimetabolites, such as sulfonamides, trimethroprim and daspone. They interfere with the metabolic pathways of the microrganisms.
According to their spectrum of activity, antibiotics are divided into narrow-spectrum (against a restricted number of microrganisms) and broad-spectrum (against a larger number of microrganisms).

17. Combination of antimicrobial chemotherapeutics and its in vitro activity testing

18. Resistance of microorganisms to antimicrobial chemotherapeutics and its transfer

Resistance of a microrganism to a antimicrobial chemotherapeutic is the ability to survive in presence of that antibiotic. It’s a phenomenon relatively new to medicine and is the result of years of application of the same antibiotics, leaving bacteria able to produce this resistance towards them.
Resistance can be classified into natural or acquired. The second type can be due to enzymes of the bacteria, changes in the site of action, eflux of the drugs back out of the bacteria after penetration, changes in permeability of bacterial walls and biofilms.
Acquired resistance usually occurs with plasmids, small pieces of circular, double-stranded DNA, intoduced into bacteria by various means (conjugation, transduction etc). They contain genetic information in their genes that codes for antibiotic-resistant enzymes.



19. Penicillins

Penicillin antibiotics are very effective antibiotics with an extremely low toxicity. The basic compound is an organic acid with a beta-lactam ring obtained from culture of the mold Penicillium chrysogenum. The biochemical modification of the 6-aminopenicillanic acid produced by these molds in fermentation results in decreased acid lability and increased absorption in the gastrointestinal tract, resistance to destruction by penicillinases or a broader spectrum of activity that includes gram-negative bacteria.
Penicillin G is incompletely absorbed because it's inactivated by gastric acid. Thus it's used as an intravenous drug for the treatment of infections caused by the limited number of susceptible organisms. Penicillin V is more resistant to acid is the preferred oral form for the treatment of susceptible bacteria. Penicillinase-resistant penicillins such as methicillin and oxacillin are used to treat infections caused by susceptible staphylococci. Ampicillin was the first broad-spectrum penicillin, although the spectrum of the activity against gram-negative rods was limited primarily to Escherichia, Proteus and Haemophilus species.
Selected penicillins have been combined with beta-lactamase inhibitors. These have shown to be relatively effective in treating certain infections caused by beta-lactamase producing bacteria.

20. Cephalosporins, carbapenems and monobactams

The cephalosporins are beta-lactam antibiotics, originally isolated from the mold Cephalosporium. Cephalosporins have the same mechanism of action as penicillins. However, they have a wider antibacterial spectrum, are reistant to many beta-lactamases and have improved pharmacokinetic properties (e.g. longer half-life).
Biochemical modifications to the cephalosporin molecule have made them have enhanced activity against gram-negative bacteria compared to penicillins. The activity of narrow-spectrum first generation cephalosporins is directed primarily against Escherichia coli, Klebsiella species, Proteus mirabilis and oxcacillin-susceptible gram-positive cocci. Many of the expanded-spectrum second generation cephasporins have additional activity against Haemophilus influenzae, Enterobacter, Citrobacter and Serratia species and some anaerobes such as Bacteroides fragilis. The broad-spectrum third generation cephalosporins are active against most Enterobacteriaceae and Pseudomonas aeruginosa.
Carbapenems are important, widely-perscribed broad-spectrum antibiotics that are active against virtually all groups of organisms, with only a few exceptions (e.g. Pseugomonas and gram-positive rods). In contrast, monobactams are narrow-spectrum antibiotics, active against only aerobic, gram-negative bacteria. This is advantageous when one wants to treat a patient without disrupting the normal protective flora. Despite this, monobactams are not widely used.

21. Tetracyclines and chloramphenicols

Tetracyclines are broad-spectrum bacteriostatic antibiotics that inhibit protein synthesis in bacteria by binding reversibly to the ribosomal 30S subunit. Tetracyclines are effective in the treatment of infections caused by Chlamydia, Mycoplasma and

Rickettsia species and other selected gram-positive and gram-negative bacteria. All tetracyclines have a similar spectrum of activity, with the primary difference among the antibiotics being in their pharmacokinetic properties.
Resistance to the tetracyclines can stem from decreased penetration of the antibiotic into the bacterial cell, active efflux of the antibiotic out of the cell, alteration of the ribosomal target site or enzymic modification of the antibiotic.
The most common cause of resistance to tetracyclines are mutations in genes coding for proteins that control active efflux of the antibiotics. Resistance can also result from the production of proteins similar to elongation factors that protect the 30S ribosome.
Chloramphenicol has a broad antibacterial spectrum similar to that of tetracycline but is not commonly used. The reason is that besides interfering with bacterial protein synthesis, it disrupts protein synthesis in human bone marrow cells and can produce blood dyscrasias such as aplastic anaemia. Chloramphenicol acts by reversibly binding to the peptidyl transferase component of the 50S ribosomal subunit, thus blocking peptide elongation.
Resistance to chloramphenicol is observed in bacteria producing plasmid-encoded chloramphenicol acetyltransferase, thus making the product incapable of binding to the 50S subunit.

22. Aminoglycosides

The aminoglycoside antibiotics consist of amino sugars linked through glycosidic bonds to an aminocyclitol ring. Streptomycin, neomycin, kanamycin and tobramycin were originally isolated from Streptomyces species and gentamicin and sisomicin from Micromonospora species. These antibiotics exert their effect by passing through the bacterial outer membrane, cell wall and cytoplasmic membrane to the cytoplasm where they inhibit bacterial protein synthesis by binding irrerersibly to the 30S subunit of bacterial ribosomes. This attachment has two effects: production of aberrant proteins as the result of misreading of the mRNA and interruption of protein synthesis by causing the premature release of the ribosome from the mRNA.
Aminoglycosides are bactericidal because of their ability to bind irreversibly to ribosomes and are commonly used to treat serious infections caused by many gram-negative rods (Enterobacteriaceae, Pseudomonas, Acinetobacter) and some gram-positive organisms. Anaerobes are resistant to aminoglycosides because the antibiotics disrupt aerobic processes of bacterial metabolism. Streptococci and enterococci are resistant to aminoglycosides, because the antibiotics fail to penetrate through the cell wall of these bacteria.
The most commonly used antibiotics in this class are amikacin, gentamicin and tobramicin. All three are used to treat serious infections from susceptible gram-negative bacteria. Amikacin has the best activity and is frequently reserved for treatment of infections caused by gram-negative bacteria and are resistant to gentamicin and tobramicin. Streptomycin is not readily available but has been used for the treatment of tuberculosis, tularemia and gentamicin-resistant streptococcal or enterococcal infections.
Resistance to aminoglycosides can develop in on of four ways: mutation of the ribosomal binding site, decreased uptake of the antibiotic into the bacterial cell, increased expulsion of the antibiotic from the cell or enzymatic modification of the

antibiotic.

23. Macrolides, lincosamides and polypeptidic antibiotics.

Erythromycin, derived from Streptomyces erythreus, is the model macrolide antibiotic. Modification of the macrolide structure led to the development of azithromycin and clarithromycin. Macrolides act by reversibly binding to the 23S rRNA of the 50S ribosomal subunit, which blocks polypeptide elongation. Resistance to macrolides most commonly stems from the methylation of the 23S rRNA, preventing binding of the antibiotic. Macrolides are bacteriostatic antibiotics with a broad spectrum of activity. They have been used to treat pulmonary infections caused by Mycoplasma, Legionella and Chlamydia, as well as treat infections caused by Campylobacter species and gram-positive bacteria in patients with allergies to penicillin. Most gram-negative bacteria are resistant to macrolides.
Lincosamides, such as lincomycin and clindamycin, are a class of antibiotics that are bacteriocidal by interfering with bacterial protein synthesisthey bind to the 23S rRNA of the 50s ribosomal subunit, like macrolides. They don't interfere with cellular protein synthesis since bacterial ribosomes are structurally different. The first lincosamide was isolated from the bacterium Streptomyces lincolnensis. Clindamycin is a very good antibacterial agent and has replaced the use of lincomycin. Clindamycin also exhibits some activity against parasitic protozoa that cause toxoplasmosis and malaria. Lincosamides are used mostly to treat infections from Streptococci and Staphylococci. Resistance may rise from changing of the bacterial binding site but on rare occasions, enzymatic inactivation of clindamycin is observed.
Bacitracin, which was isolated from Bacillus licheniformis, is a mixture of polypeptides used in tropically applied products for the treatment of skin infections caused by gram-positive bacteria (especially Staphylococci). Gram-negative bacteria are resistant to this agent. Bacitracin inhibits cell wall synthesis by interfering with dephosphorylation and the recycling of the lipid carrier responsible for moving the peptidoglycan precursors through the cytoplasmic membrane to the cell wall. Resistance to the antibiotic is most likely caused by failure of the antibiotic to penetrate into the bacterial cell.
Polymyxins are a group of cyclic polypeptides derived from Bacillus polymyxa. These antibiotics insert into bacterial membranes like detergents by interacting with lipopolysaccharides and the phospholipids in the outer membrane, producing increased cell permeability. Polymyxins B and E (colistin) are capable of causing serious nephrotoxicity. Thus their use is limited mainly to external infections. Oral administration is used to sterilise the gut. These antibiotics are most effective against gram-negative rods, because gram-positive bacteria lack an outer membrane.

24. Antituberculotics

Antituberculotics are antibiotics against the acid-fast bacteria, mycobacteria.
Clofazimine is a lipophilic antibiotic that binds to mycobacterial DNA. It's highly active against Mycobacterium tuberculosis, it's a first-line drug for the treatment of Mycobacterium leprae infections and has been recommended as a secondary antibiotic

for the treatment of infections caused by other mycobacterial species.
Pyrazinamide is active against Mycobacterium tuberculosis at low pH, such as that found in phagolysosomes. The active form of this antibiotic is pyrazinoic acid, produced when pyrazinamide is hydrolysed in the liver. The mechanism of action of pyrazinamide is unknown.

25. Sulfonamides and imidazoles

The sulfonamides are antimetabolites that compete with p-aminobenzoic acid, thereby preventing synthesis of folic acid required by certain microorganisms. Because mammals don't synthesise folic acid, sulfonamides don't interfere with their cell metabolism.
Sulfonamides are effective against a broad range of gram-positive and gram-negative organisms, such as Nocardia, Chlamydia and some protozoa. Short-acting sulfonamides such as sulfisoxazole are among the drugs of choice for the treatment of acute urinary tract infections caused by susceptible bacteria such as Escherichia coli.
Resistance to these antibiotics can stem from a variety of mechanisms. Bacteria such as Pseudomonas are resistant as the result of permeability barriers. A decreased affinity of dihydrofolate reductase can be the source of trimethoprim resistance. Also, bacteria tha use exogenous thymidine (e.g. enterococci) are also intrinsically resistant.
Imidazoles belong to the class of azole antifungal agents, including ketoconazole, miconazole and clotrimazole. Imidazoles act by inhibiting the fungal enzyme lanosterol-14-alfa-demethylase, necessary for conversion of lanosterol to ergosterol. Depletion of lanosterol in the fungal membrane disrupts its structure and many of its functions leading to inhibition of fungal growth, thus making imidazole fungostatic rather than fungocidal.

26. Quinolones, nitrofurans and topical antibacterial chemotherapeutics

The quinolones are one of the most widely used classes of antibiotics. These are synthetic chemotherapeutic agents that inhibit bacterial DNA topoisomerase type II (gyrase) or type IV, which are required for DNA replication, recombination and repair. The DNA gyrase-A subunit is the primary targer of quinolones in gram-negative bacteria, whereas topoisomerase IV is that in gram-positive bacteria. The first quinolone used was nalidixic acid. It was used to treat urinary tract infections caused by a variety of gram-negative bacteria but resistance developed rapidly. Today, this drug has been replaced by newer quinolones, such as ciprofloxacin, levofloxacin and others. These are also referred to as fluoroquinolones. Resistance to these antibiotics can develop rapidly in Pseudomonas, oxacillin-resistant staphylococci and enterococci.
Resistance to quinolones is mediated by chromosomal mutations in the structural genes for DNA gyrase and topoisomerase IV. Other mechanisms include overexpression of efflux pumps.

27. Antiviral chemotherapeutics

Most of the antiviral drugs approved are nucleoside analogues that inhibit viral polymerases. Resistance to the drug is usually caused by a mutation of the polymerase. Acyclovir (acycloguanosine) differs from the nucleoside guanosine by having an acyclic side chain instead of a ribose or deoxyribose sugar. Acyclovir has selective action against HSV and VZV, the herpesviruses that encode a thymidine kinase. Resistance to acyclovir develops by mutation of either the thymidine kinase, or the DNA polymerase, to prevent acyclovir binding.
Valacyclovir, the valyl ester derivative of acyclovir, is more effectively absorbed after oral administration and rapidly converted into acyclovir.
Penciclovir inhibits HSV and VZV in the same way acyclovir does but is concentrated and persists in the infected cells to a greater extent that acyclovir. It also has some activity against the Epstein-Barr virus and cytomegalovirus.
Famciclovir is a prodrug derivative of penciclovir that is well absorbed orally and then is converted to penciclovir in the liver and intestinal lining.
Ganciclovir differs from acyclovir in having a single hydroxymethyl group in the acyclic side chain. The result of this addition is that it confers considerable activity against cytomegalovirus. Ganciclovir is effective against cytomegalovirus retinitis and shows some efficacy in the treatment of cytomegalovirus esophagitis, colitis and pneumonia in patients with AIDS.
Apart from nucleoside analogues, there are also non-nucleoside polymerase inhibitors. Foscarnet and the related phosphoacetic acid are simple compounds that resemble pyrophosphate. These drugs inhibit viral replication by binding to the pyrophosphate-binding site of the DNA polymerase to block nucleotide binding. It doesn’t inhibit cellular polymerases at pharmacologic concentrations but it can cause renal and other problems because of its ability to chelate divalent metal ions (e.g. calcium) and become incorporated in bone. Foscarnet inhibits DNA polymerase of all herpesviruses and the HIV reverse transcriptase without having to be phospharylated by nucleoside kinases.
The unique structure of HIV protease and its essential role in the production of a functional virion have made proteases a good target for antiviral drugs. Saquinavir, indinavir and other work by slipping into the hydrophobic active site of the enzyme to inhibit its action. Drug-resistant strains arise through mutations with azidothymine and a second nucleoside analogue (HAART) can reduce blood levels of HIV to undetectable.

28. Antimycotic chemotherapeutics

Amphotericin B and its lipid formulations are polyene macrolide antifungals used in the treatment of serious life-threatening mycoses.
Amphotericin B exerts its antifungal action by at least two different mechanisms. The primary one involves the binding of amphotericin B to ergosterol, the principal membrane sterol of fungi. This binding produces ion channels, which destroy the osmotic integrity of the fungal cell membrane and lead to leakage of intracellular constituents and cell death. Amphotericin B also binds cholesterol, the main sterol of mammalian cells, but does so less avidly than to ergosterol. This accounts for most of the toxicity observed when amphoterisin B is administered to humans.

The spectrum of activity of Amphotericin B is broad and includes most strains of Candida, Cryptococcus neoformans, Aspergillus species, the zygomycetes and the endemic dimorphic pathogens (Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum). Aspergillus terreus, Fusarium species and certain dematiaceous fungi may be resistant to amphotericin B. Resistance has been associated with alterations in membrane sterols, usually a reduction in ergosterol.
Amphotericin B is widely distributed in various tissues and organs, including the liver, kidneys, bone marrow and lungs. Although negligible concentrations of Amphotericin B can be found in the CSF, it's generally effective in treating infections of the CNS.
Amphotericin B is considered fungicidal against most fungi.
The azole class of antifungals may be divided in terms of structure into the imidazoles and the triazoles. Among imidazoles, only ketoconazole has systemic activity. The triazoles all have systemic activity and include fluconazole, itraconazole, voriconazole and posaconazole.
Both imidazoles and triazoles act by inhibiting the fungal cytochrome p450-dependent enzyme lanosterol 14-a-demethylase. Inhibition of ergosterol synthesis disrupts membrane synthesis in the fungal cell which results in inhibition of fungal cell growth (fungistatic) or cell death (fungicidal). Most azole exhibit fungistatic activity against Candida species and Cryptococcus neoformans.
Ketoconazole is an orally absorbed, lipophilic member of the imidazole class of antifungal agents. Its spectrum of activity includes the endemic dimorphic pathogens, Candida species, Cryptococcus neoformans and Malassezia species, although it's generally less active than the triazole antifungal agents. Zygomycetes and Aspergillus species are resistant.
Fluconazole is a first-generation triazole with excellent oral bioavailability and low toxicity. It's used extensively and is active against most Candida species, Cryptococcus neoformans, dermatophytes, Trichosporon species, Histoplasma capsulatum and others. It's water-soluble and can be administered either orally or intravenously. Protein binding is low and the drug is distributed to all organs and tissues.
Echinocandins are a novel, highly selective, class of semisynthetic lipopeptides that inhibit the synthesis of 1,3-beta-glucans, important constituents of the fungal cell wall. Since mammalian cells don't contain this, this class of agents is selective in its toxicity for fungi in which the glucans play an important role in maintaining the osmotic integrity of the fungal cell.
The spectrum of activity of echinocandins is limited to those fungi where 1,3-beta-glucans constitute the domain cell wall glucan component. These are Candida and Aspergillus species and have various activity against dematiaceous fungi. They are inactive against Cryptococcus neoformans, Trichosporon species and others.
Flucytosine is the only available antifungal agent that functions as an antimetabolite. It's a fluorinated pyrimidine analogue that exerts antifungal activity by interfering with the synthesis of DNA, RNA and proteins in the fungal cell.
The antifungal spectrum of flucytosine is limited to Candida species, Cryptococcus neoformans, Rhodotorula species and selective dematiaceous molds. Althoigh primary resistance to flucytosine is rare among isolates of Candida species, resistance may develop among Candida and Cryptococcus neoformans during flucytosine monotherapy.
A variety of topical antifungal preparations is also available for the treatment of

superficial cutaneous and mucosal fungal infections. Topical antibiotics are available for almost all classes of antifungal agents. Onychomycoses are bestly treated with ointments, creams, lotions, powders and sprays whereas mucosal infections with tablets and suspensions.

29. Antiparasitic chemotherapeutics

The number of antiparasitic agents is much smaller than that of antimicrobial agents, although the goal is similar: to eradicate the organism rapidly and completely. Immunocompromised individuals pose a particular problem with respect to antiparasitic chemotherapy. Prophylaxis is effective against preventing an infection, such as that from toxoplasma. However, once an infection is established, radical cure may not be possible and long-term supressive therapy may be indicated.
Antiprotozoal agents, similar to antifungal and antimicrobial agents, are generally targeted at relatively rapidly proliferating, young, growing cells.
Heavy metals are used for the therapy of parasitic infections including arsenical (melarsoprol) and antimonial compounds (sodium stibogluconate). These agents are thought to oxidise certain groups of enzymes necessary for carbohydrate metabolism.
Quinoline derivaties include chloroquine, quinine, quinidine, primaquine and synthetics such as mefloquine. These all have antimalarial activity abd accumulate preferentially in parasitised red blood cells. Mechanisms of action include binding to DNA and interfering with DNA replication and binding to ferriprotoporphyrin IX released from haemoglobin in infected erythrocytes, producing a toxic complex.
Folic acid antagonists are deadly against parasites, because they are unable to absorb exogenous folate and thus are susceptible to drugs that inhibit its synthesis. Folic acid antagonists may be pyrimethamine and sulfonamides. These compounds block separate steps in the folic acid pathway. When a diaminopyrimidine is used with a sulfonamide, they have an synergistic effect.
Clindamycin and tetracyclines can be used against Plasmodium species, Babesia species and amoebae, acting as protein synthesis inhibitors. Doxycycline is used against the chloroquine-resistant Plasmodium falciparum malaria.
Nitroimidazoles include the well-known antibacterial agent metronidazole. The mechanism of action suggests that it inhibits DNA and RNA synthesis and also inhibits the metabolism of glucose.
The strategy of the use of antihelmintic drugs is quite different from that of antiprotozoal drugs. Most antihelmintic drugs are targeted at non-proliferating adult organisms.
Benzimidazoles are broad-spectrum antihelmitic agents. They inhibit fumarate reductase, glucose transport, resulting in glycogen depletion and disruption of mitochondrial function. The spectrum of activity includes intestinal nematodes (Ascaris, Trichuris, Necator) as well as a number of cestodes (Taenia, Hymenolepis).
Tetrahydropyrimidines, such as pyrantel pamoate, are cholinergic agonists that have a powerful effect on nematode muscle cells by binding to cholinergic receptors, resulting in cell depolarisation and muscle contraction. This paralytic action leads to expulsion of the worm from the host intestinal tract.
The piperazine antihelmitic agent used most commonly is diethylcarbamazine. It acts in

a way similar to tetrahydropyrimidines, by binding to cholinergic receptors and causing muscle contraction. It also enhances the adherence of leukocytes to microfilariae and thus may act by altering the parasite surface membrane or directly stimulating phagocytic cells.
Avermectins, such as ivermectin, act by interacting with the chloride channel in nerve and muscle cell membranes, resulting in hyperpolarisation of the affected cells and consequent paralysis and death of the parasites. The drug also inhibits the reproductive function of the adult female Onchocerca volvulus and alters the ability of its microfilariae to evade the host immune system. In humans it's used mostly only in treatment of ocular and lymphatic filariasis.

30. Disinfection and sterilisation (methods and agents)

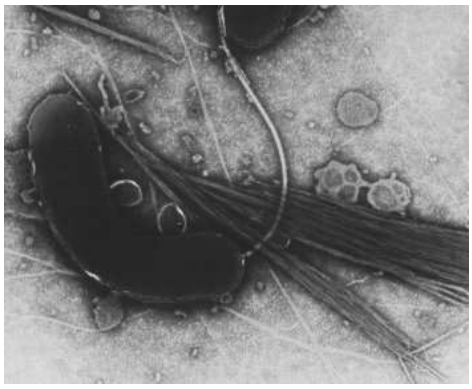
Sterilisation is the total destruction of all microbes, including the more resilient forms such as bacterial spores, mycobacteria, nonenveloped viruses and fungi.
Physical sterilants such as moist and dry heat, are the most common sterilising methods used in hospitals and are indicated for most materials, except those that are heat sensitive. Filtration is useful for removing bacteria and fungi from air or from solutions. Sterilisation by ultraviolet or ionizing radiation is also commonly used.
Ethylene oxide is the most commonly used gas vapor sterilant. Although it's highly efficient, it's also highly flammable. Hydrogen peroxide vapors are effective sterilants because of the oxidising nature of the gas. A variation is plasma gas sterilisation, in which hydrogen peroxide is vaporised and then reactive free radicals are produced by either microwave-frequency or radio-frequency energy.
Two chemical sterilants have also been used, peracetic acid and glutaraldehyde. Peracetic acid, an oxidising agent, has excellent activity and the end products are non-toxic. In contrast, safety is a concern with glutaraldehyde and care must be used when handling this chemical.
Microbes are also destroyed by disinfection procedures, although more resilient organisms can survive.
High-level disinfectants are used for items involved with invasive procedures that can't withstand sterilisation procedures. Disinfection of these items is most effective if cleaning the surface to remove organic matter precedes treatment. Examples are moist heat and use of liquids such as glutaraldehyde, hydrogen peroxide etc.
Intermediate-level disinfectants (alcohols, iodophor compounds, phenolic compounds) are used to clean surfaces or instruments in which contamination with bacterial spores and other highly-resistant organisms is unlikely.
Low-level disinfectants (quaternary ammonium compounds) are used to treat non-critical instruments and devices such as blood pressure cuffs, electrocardiogram electrodes and stethoscopes.
The level of disinfectants used for environmental surfaces is determined by the relative risk these surfaces pose as a reservoir of pathogenic organisms.

Special Microbiology

1. Vibrio cholerae and other vibrios, Aeromonas, Plesiomonas

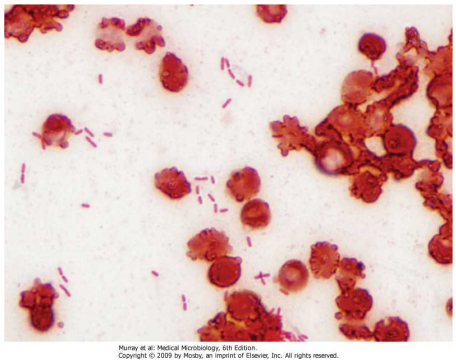
The genera Vibrio and Aeromonas belong to the group of gram-negative, facultatively anaerobic, fermentative rods.
The genus Vibrio is currently composed of 76 species of curved rods. Vibrios grow on a variety of simple media within a broad temperature range (14-40 ^{o}C). All species of vibrios require salt to grow. Vibrio cholerae can grow on most media without added salt, but most others require addition of NaCl. Vibrios tolerate a wide range of pH (6,5-9) but are susceptible to stomach acids. Most vibrios have polar flagella as well as various pili that are important for virulence. All strains contain the lipopolysaccharide of lipid A (endotoxin). Vibrio cholerae O1 and O139 produce the cholera toxin and are associated with epidemics of cholera. Other vibrios include Vibrioparahaemolyticus and Vibriovulnificus.

The bacteriophage CTXΦ encodes the genes for the two subunits of cholera toxin. This bacteriophage binds to the toxin co-regulated pilus and moves into the bacterial cell, where it becomes integrated into the Vibrio cholerae genome.
The majority of individuals exposed to toxigenicVibriocholerae O1 have asymptomatic infections or self-limited diarrhea. However, some individuals develop severe, rapidly fatal diarrhea. Disease caused by Vibrio cholerae O139 can be as severe as disease caused by Vibrio cholerae O1. Other serotypes of Vibrio cholerae (non-O1) don't contain the cholera toxin and are responsible only for mild watery diarrhea.



The severity of gastroenteritis caused by Vibrio parahaemolyticus can range from self-limited diarrhea to a mild, cholera-like illness. Wound infection with this organism can occur in people with exposed to contaminated seawater.
Vibrio vulnificus is a particularly virulent species of Vibrio responsible for more than 90% of the vibrio-related deaths. Vibriovulnificus infections are most severe in patients with hepatic diseases, hematopoietic diseases or chronic renal failure and in those receiving immunosuppressive drugs.
Patients with cholera must be promptly treated with fluid and electrolyte replacement before the resultant massive fluid loss leads to hypovolemic shock. Azithromycin is the drug of choice for children and adults. Although long-term carriage of Vibrio cholerae doesn't occur, vibrios are free living in estuarine and marine environments. Only improvements in sanitation can lead to control of the disease. Also, a variety of cholera vaccines have been developed with none providing long-term protection.
Aeromonas is a gram-negative, facultatively anaerobic fermentative rod that morphologically resembles members of the family Enterobacteriaceae. The most important pathogens are Aeromonashydrophila, Aeromonascaviae and Aeromonasveronii.
Aeromonas species cause three forms of disease, diarrheal disease in otherwise healthy people, wound infections and opportunistic systemic disease in immunocompromised patients. Gastroenteritis typically occurs after the ingestion of contaminated water or food whereas wound infections commonly result from a traumatic injury associated with exposure to contaminated water.
Ciprofloxacin is constituently active against Aeromonas strains howeverresistance is being developed. Thus, the long-term effect of fluoroquinolones is yet to be seen. Gentamicin and amikacin are also effective against aeromonads. Aeromonas species are resistant against penicillins, most cephalosporins and erythromycin.

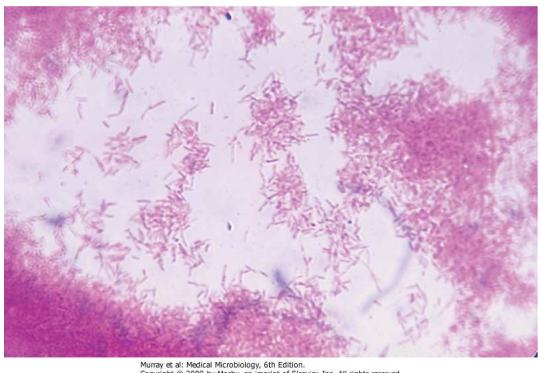
2. Pseudomonadaceae, other gram-negative non-fermenting bacteria



Pseudomonas and related non-fermentative rods are a mixture of opportunistic pathogens of plants, humans and animals. Despite the many genera, most clinically significant isolates are members of five genera: Pseudomonas, Burkholderia, Stenotrophomonas, Acinetobacter and Moraxella.
In the genus Pseudomonas, the most important species is Pseudomonas aeruginosa. Members of this genus are ubiquitous, found in soil, decaying organisms and water. Pseudomonas infections are primarily opportunistic (restricted to patients receiving broad-spectrum antibiotics that suppress normal intestinal flora or areimmunocompromised).
Pseudomonas species are usually motile, straight or slightly curved, gram-negative rods, typically arranged in pairs. They utilize carbohydrates through aerobic respiration. The presence of cytochromeoxidase in Pseudomonas is used to differentiate them from Enterobacteriaceae.
Pseudomonas aeruginosa has many virulence factors, including structural componets (flagella, pili, LPS, alginat), toxins (exotoxin A, pyocyanin) and enzymes (serine protease, metalloprotease, PLC). Also, the delivery system used by Pseudomonas aeruginosa type III secretion system, is particularly effective in injecting toxins into the host cell. It has been thought that multiple factors must work together for Pseudomonas aeruginosa to cause disease.
Pseudomonas aeruginosa is inherently resistant to many antibiotics and can mutate to even more resistant strains during therapy. Pseudomonas aeruginosa also produces many beta-lactamases to inactivate beta-lactam antibiotics like penicillins, cephalosporins and carbapenems.
Pseudomonas aeruginosa infections of the lower respiratory tract can vary from asymptomatic colonization to necrotizing bronchopneumonia. Infections of the skin and soft tissues are also common (burn wounds, folluculitis, osteochondritis), along with urinary tract infections, ear and eye infections and also endocarditis.
Pseudomonas has simple nutritional requirements so its culture is readily recovered on common isolation media such as blood agar and MacConkey agar. During beta-haemolysis tests, a green pigmentation is produced due to pyocyanin and pyoverdin.
A combination of active antibiotics is generally required for therapy to be successful. Effective infection-control practices should concentrate on preventing the contamination of sterile equipment.

The most important species of the Brukholderia genus is Brukholderiacepacia. It also colonises a variety of moist environments and is opportunistically pathogenic. Patients particularly susceptible to pulmonary infections are those with cystic fibrosis or chronic granulomatous disease.Brukholderiacepacia complex is also responsible for urinary tract infections in catheterized patients, septicemia and other opportunistic infections. Brukholderia species are susceptible to trimethoprim-sulfamethaxazole, which distinguishes them from Pseudomonas aeruginosa.
Stenotrophomonasmaltophilia is the most important species of the Stenotrophomonas genus. It's responsible for infections in debilitated patients with impaired host-defense mechanisms. Also, because it's resistant to most commonly used beta-lactam and aminoglycoside antibiotics, patients receiving long-term therapy with these drugs are particularly at risk. The most common infections are bacteremia and pneumonia due to contaminated equipment. Antimicrobial therapy is complicated because of the high degree of resistance. Trimethoprim-sulfamethoxazole is the agent most active in therapy.
Acinetobacters are strictly aerobic, oxidase-negative, gram-negative coccobacilli. They are ubiquitous saprophytes, recovered in nature and in the hospital and are able to survive on both moist and dry surfaces. They are also part of the normal oropharygneal flora of a small number of people. The genus acinetobacter is subdivided into two groups: glucose-oxidising (Acinetobacterbaumannii) and glucose non-oxidising (Acinetobacterhaemolyticus). Acinetobacters are opportunistic pathogens that cause infections in the respiratory tract, urinary tract and wounds. Patients most at risk of infection are those being treated with broad-spectrum antibiotics post-surgery or on respiratory ventilation. Treatment is difficult because the organisms are often resistant.
Moraxellacatarrhalis is the most important species of the Moraxella genus. It's astrictly aerobic, oxidase-positive, gram-negative diplococcus. It's a common cause of bronchitis and bronchopneumonia. Most isolates produce beta-lactamases for resistance against penicillins. However, these bacteria are susceptible to most other antibiotics. Human colonizing species include Moraxellaosloensis and Moraxellanonliquefaciens, found on the skin and mucosa of the mouth and genitourinary tract. They're a rare cause of opportunistic infections.

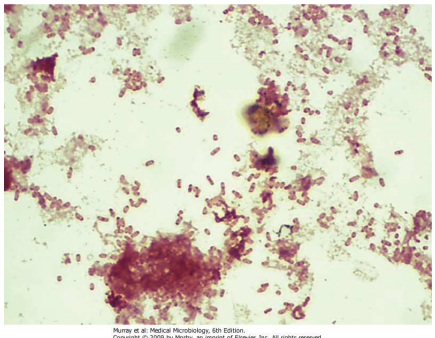
3. Legionellaceae



Studies have shown that the family Legionellaceae contains the genus Legionella with 50 species. About half have been identified as human pathogens. Legionella

pneumophila is the cause of 90% of all infections. Serotypes 1 and 6 are most common. Members of the genus Legionella are slender, pleomorphic, gram-negative rods. They appear as short coccobacilli when observed in tissue. Legionellae don't stain with common reagents but can be seen in tissues stained by Dieterle silver stain. Legionellae are obligate aerobes and nutritionally fastidious. They require cysteine and iron for primary isolation. They don't grow on conventional blood agar media. Respiratory tract diseases caused by Legionella species develop in susceptible people who inhale infectious aerosols. Legionellae are facultative intracellular parasites that multiply in free-living amoebae in nature and in alveolar macrophages, monocytes and alveolar epithelial cells. The replicative cycle starts by binding to the C3b component of the complement system on the macrophage membrane, through CR3 receptors. The bacteria aren't killed because the phagolysosome is inhibited. Chemokines and cytokines are released, causing an inflammatory reaction, typical of Legionella infection. Production of interferone-γ is critical for elimination of Legionella organisms. Asymptomatic Legionella infections are thought to be very common. Symptomatic infections primarily affect the lungs and present in one of two forms: an influenza-like illness (Pontiac fever) and a sever form of pneumonia (legionnaires disease). Legionellosis caused by Legionella pneumophila serogroup 1 is commonly diagnosed with the use of enzyme immunoassays or indirect fluorescent antibody tests. Macrolides or fluoroquinolones are the most effective antibiotics against Legionella genus infections. Prevention requires identification of the environmental source of the organism and reduction of the microbial burden (e.g. hyperchlorination of the water supply).

4. Salmonella typhi and Salmonella paratyphi

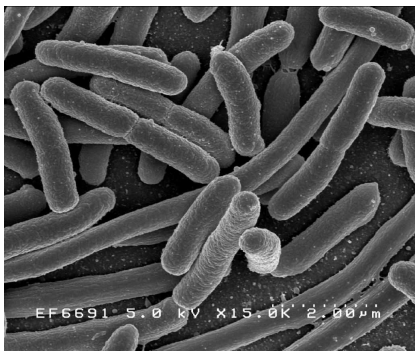


Salmonella genus belongs to the family Enterobacteriaceae. There are more than 2,500 unique serotypes belonging to Salmonella enterica but three are commonly listed as

Shigella dysenteriae, Shigella flexneri, Shigella boydii and Shigella sonnei.
Shigellae cause disease by invading and repliacting in cells lining the colon. This activity is mediated by structural gene proteins within a plasmid, under chromosomal control.
Shigella species appear unable to attach to differentiated mucosal cells, rather, they first attach to and invade the M cells located in Peyers patches. Shigellae, after being endocytosed, lyse the vacuole and replicate in host cell cytoplasm. Through the cell's actin filaments, bacteria propell them selves from cell to cell, avoiding immune-mediated clearance. Shigellae survive phagocytosis by inducing apoptosis.
Shigella dysenteriae strains produce an exotoxin, Shiga toxin, consisting of one A subunit and five B subunits. The A subunit binds to the ribosomal subunits of the cell, preventing protein synthesis. Primary manifestation of toxin activity is damage to intestinal epithelium.
Shigellosis is characterised by abdominal cramps, diarrhea, fever and bloody stools.
The infection is generally self-limited although antibiotic treatment can reduce the risk of secondary spread to people around the patient.
The specimen used for laboratory diagnosis of Shigella is stool.
Shigella organisms are predominantly transmitted in young children. The best way to prevent this is only through proper education, teaching handwashing and proper disposal of soiled diapers.

7. Escherichia coli

Escherichia coli, belonging to the Enterobacteriaceae family, is the most common and important member of the Escherichia genus.
In addition to the general factors possessed by all members of the Enterobacteriaceae family, Escherichia coli possess specialised virulence factors such as adhesins and exotoxins.

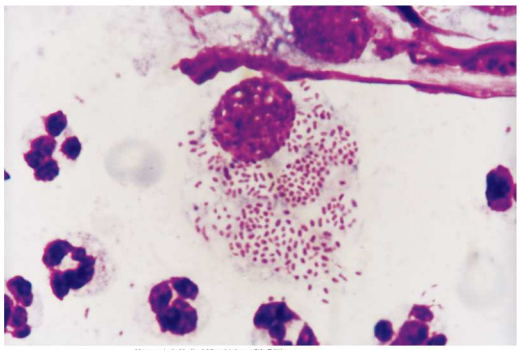


Escherichia coli is responsible for a variety of diseases, such as gastroenteritis, urinary tract infections, meningitis and sepsis.
Strains of Escherichia coli that cause gastroenteritis are subdivided into 5 subgroups: enterotoxigenic, enteropathogenic, enteroaggregative, enteroinvasive and enterohaemorrhagic.
The first three cause secretory diarrhea involving the small intestine and the others primarily involve the large intestine.
Initially, the bacteria produce watery diarrhea and a minority of patients progress to dysenteric form of disease, consisting of fever, cramps and blood in stool.
Urinary tract infections occur when the bacteria, that originate in the colon, contaminate the urethra, ascend to the bladder and may migrate to the kidneys or prostate.
Escherichia coli and subgroup B streptococci cause the majority of CNS infections in infants younger than 1 month and is due to the K1 capsular antigen, leading to bacterial meningitis.
Immunocompromised patients have a higher mortality rate than patients with a normal immune system, associated with septicemia.
Detection of Escherichia coli in the laboratory can be done by one of two ways: culture and toxin detection. Sorbitol-containing MacConkey agar is used for conformation of sorbitol-negative, gram-negative bacteria, serotype O157, in stool specimens.
It is difficult to prevent infection with Escherichia coli, because this organism is part of the normal intestinal flora. However, some risk factors should be avoided, such as unrestricted use of antibiotics that can select for resistant bacteria or the use of urinary catheters.

8. Proteus, Klebsiella, Enterobacter, Citrobacter and other genera of Enterobacteriaceae family

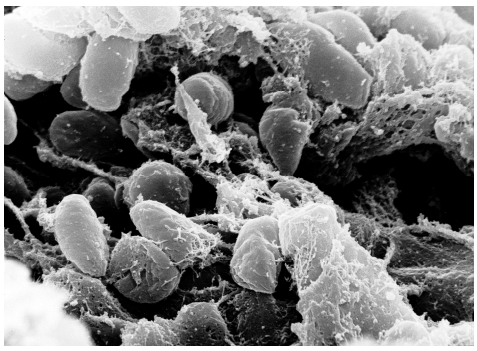
Proteus, Klebsiella, Enterobacter and Citrobacter belong to the family of Enterobacteriaceae and are gram-negative rods.
Infections of the urinary tract with Proteus mirabilis are the most common diseases produced by this genus. Proteus mirabilis produces urase which splits urea into carbon dioxide and ammonia. This process raises the urine pH, precipitating magnesium and

calcium in the form of crystals resulting in the formation of renal stones. The increased alkalinity of urine is also toxic to the uroepithelium. Proteus also produces six different fimbriae, some of which are important for adherence to the uroepithelium.



Members of the Klebsiella genus have a prominent capsule that is responsible for the mucoid appearance of isolated colonies and their enhanced virulence. The most commonly isolated members of this genus are Klebsiella pneumoniae and Klebsiella oxytoca, which cause primary lobar pneumonia. Pneumonia caused by Klebsiella species usually involves necrotic destruction of alveolar spaces, formation of cavities and production of blood-tinged sputum.
There is also another genus of Klebsiella causing disease, called Klebsiella granumolatis, which causes granuloma inguinale, affecting the inguinal area and genitalia. It can be transmitted after repeated exposure through sexual intercourse or nonsexual trauma to the genitalia.
Primary infections caused by Enterobacter and Citrobacter are rare in immunocompetent patients. They are more commonly causes of nosocomial infections in neonates and immunocompromised patients. Citrobacter koseri has been recognised to have a predilection for causing meningitis and brain abscess in neonates.
Antibiotic therapy for these genera can be ineffective because the organisms are frequently resistant. Resistance is a particularly serious problem with Enterobacter species.

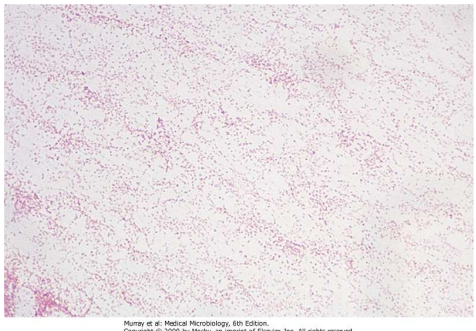
9. Yersinia



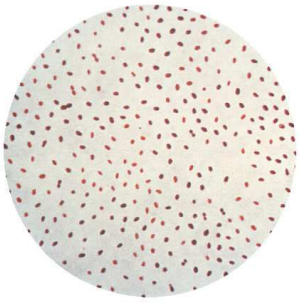
The best known human pathogens within the genus Yersinia are Yersinia pestis, Yersinia enterolitica and Yersinia pseudotuberculosis.
Yersinia pestis is a highly virulent pathogen that causes a highly fatal systemic disease known as the plague. Yersinia enterolitica and Yersinia pseudotuberculosis are primary enteric pathogens that are rarely cultured from blood.
A common characteristic of pathogenic Yersinia species is their ability to resist phagocytic killing. On contact with phagocytes, the bacteria secrete proteins into the cell that dephosphorylate several proteins required for phagocytosis.
Yersinia pestis has two plasmids that encode virulence genes: fraction 1, encoding antiphagocytic protein capsule and plasminogen activator protease gene, degrading C3b and C5a of the complement.
Two clinical manifistations of Yersinia infection are bubonic plague and pneumonic plague.
Yersinia organisms can grow at 4 ^{o}C and can multiply to high concentrations in nutritionally rich blood products that are stored in a refrigerator.
A vaccine for Yersinia pestis is no longer available, although this is likely to change in light of the concern that this ogranism can be used by bioterrorists.

10. Francisella, Brucella

Francisella and Brucella are zoonotic pathogens that occasionally cause human disease. They are fastidious and belong to very small coccobacilli. Brucella belongs to alfa-proteobacteria and Francisella to gama-proteobacteria.
The genus Francisella consists of two species, Francisella tularensis and Francisella philomiragia. The first one is the causative agent of tularemia, with rabbits being the animal carriers.
Francisella tularensis is a very small, faintly staining, gram-negative coccobacillus. It is nonmotile, has a thin lipid capsule and fastidiuous growth requirements (cysteine). It is strictly aerobic and 3 or more days of growth are required.



Francisella tularensis is an intracellular pathogen that can survive for prolonged periods in macrophages because it inhibits the phagolysosome formation. Pathogenic strains possess an antiphagocytic, polysaccharide-rich capsule, responsible for their virulence. This helps the bacteria avoid complement-mediated lysis.
Disease caused by Francisella tularensis is subdivided into several forms: ulceroglandular, oculoglandular, typhoidal, pneumonic, oropharyngeal and gastrointestinal.
The specimen collected for microbiological testing is aspirate from an ulcer or lymph node. Gram-staining is almost always unsuccessful, because of the organism's very small size. Direct staining with fluorescein-labeled antibodies against the organism is prefered. Francisella tularensis doesn't reliably grow on common laboratory media and requires a cysteine-rich environment. The best choise is cystein-enriched chocolate agar.
Streptomycin was the traditional antibiotic of choice for the treatment of all forms of tularemia. However, this antibiotic isn't readily available and is highly toxic.
Gentamicin is now considered the antibiotic of choice. Francisellae are resistant against penicillins and cephalosporins because of beta-lactamase production.



Molecular studies have shown that Brucella genus demonstrate a close relationship among the strains. Currently, there are six species of Brucella, with four of them causing disease in humans: Brucella abortus, Brucella melitensis, Brucella suis and Brucella canis. The term for the disease caused by these bacteria is brucellosis.
Brucellae are small, non-motile, non-encapsulated, gram-negative coccobacilli. They grow slowly on culture (a week or more), are strictly aerobic and non-fermentative. Colonies are both smooth and rough, according to the O antigen of the wall LPS. Brucella doesn't produce a detactable exotoxin and the endotoxin is less toxic than that produced by other gram-negative rods. The O strain of the smooth LPS is an important marker for virulence. Brucella is also an intracellular parasite of the reticuloendothelial system.
They can survive phagocytosis by macrophages by inhibiting phagolysosome formation. The disease spectrum of brucellosis depends on the infecting organism. Brucella abortus and Brucella canis tend to produce mild disease with rare suppurative complications. Brucella suis causes the formation of destructive lesions and has a prolonged course. Brucella melitensis can also cause severe disease.
Tetracyclines, with doxycycline being the best choice, are the antiotics used to treat brucellosis. However, because this is a bacteriostatic drug, relapse is common after an initially successful response. In order for treatment to be successful, it must persist for at least 6 weeks. Penicillins, fluoroquinolones and cephalosporins are ineffective.
Prevention requires vaccination of livestock.

11. Bordetella

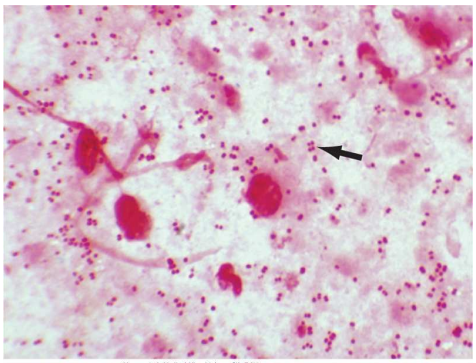
Bordetella is an extremely small, strictly aerobic, gram-negative coccobacillus. Eight species are recognised, with four causing human disease: Bordetella pertussis, Bordetella parapertussis, Bordetella bronchiseptica and Bordetella holmesii.

Bordetella species have simple nutritional requirements but some are susceptible to toxic substances and metabolites. Such species (Bordetella pertussis) require media supplemented with charcoal, starch or blood to absorb these toxic substances. They are non-motile and oxidise amino acids but are non-fermentative.
Infection with Bordetella pertussis and the development of whooping cough require exposure to the organism, bacterial attachment to the ciliated epithelium, proliferation of the bacteria and localised tissue damage and systemic toxicity.
The Pertussis toxin is a classic A-B toxin consisting of a toxic subunit (S1) and five binding subunits (S2 to S5, two S4 subunits exist). Bordetellae attach to the cilia of the respiratory tract through fimbriae.
Infection is initiated when infectious aerosols are inhaled and the bacteria attach to and proliferate on the respiratory epithelium.
Swabs enriched with calcium alginate are used for collection of specimens for microbiological testing and transport to the lab must be immediate.
Direct fluorescent antibody procedures are used to examine the specimens. PCR may also be performed.
Media used for culture include Regan-Lowe charcoal media supplemented with glycerol and horse blood. The culture should be kept at 35 ^{o}C for a week to 12 days in order to grow correctly.
Treatment of pertussis is primarily supportive, with nursing supervision during the paroxymal and convalescent stages of the illness. Macrolides (erythromycin) are effective in erradicating the organisms and can reduce the duration of infectivity.
Some strains have been reported as resistant to erythromycin but are not widespread.
Prevention includes two acellular vaccines, combined with vaccines for tetanus and diphteria which inactivate the pertussis toxin. Child administration happens in 5 doses until the age of 4 to 6 years. The adult vaccine is administered between ages 11 and 12 and then again between 19 and 65 years.

12. Haemophilus

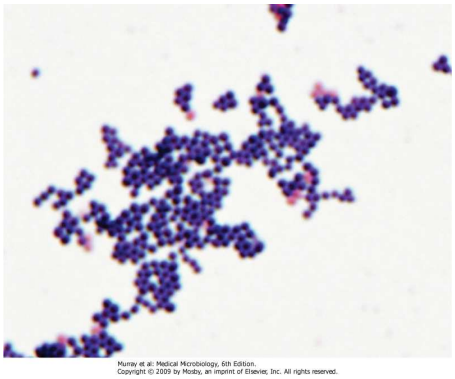
Haemophilus belongs to the family of Pasteurellaceae. It’s a small, gram-negative, facultatively anaerobic rod. Haemophilus bacteria are the most commonly isolated and significant human pathogens.
Haemophilus influenzae is the species most commonly associated with disease with

infections most often reported in pediatric patients before the Haemophilus influenzae type b vaccine. Haemophilus aegyptius is an important cause of acute conjuctivitis.
The growth of most Haemophilus species requires supplementation of media with one or both of the following growth-stimulating factors: hemin and NAD. Heated blood or chocolate agar is used for the in vitro isolation of Haemophilus.
LPS with endotoxin activity is present on the cell wall and analysis has shown a polysaccharide capsule and six antigenic serotypes.



Haemophilus species, especially Haemophilus parainfluenzae and nonencapsulated Haemophilus influenzae, colonise the upper respiratory tract in virtually all people within the first few months of life. The disease can spread and cause otitis media, sinusitis, bronchitis and pneumonia. In contrast, encapsulated Haemophilus influenzae is a common cause of disease in unvaccinated children (meningitis, epiglottitis, cellulitis).
Pili and nonpilus adhesins mediate colonisation of the oropharynx with Haemophilus influenzae. LPS and other cell wall substances inhibit ciliary function.
The major virulnce factor of Haemophilus influenzae type b is the antiphagocytic polysaccharide capsule. The LPS lipid A component induces meningeal inflammation.
Haemophilus influeznae type b is the most common cause of meningitis in children.
Direct needle aspiration should be performed for microbiological diagnosis of sinusitis or otitis and sputum produced by the lower airways is used for the diagnosis of pneumonia. Both blood and CSF are collected from nonimmune children for the diagnosis of menignitis.
Specimens, such as blood or CSF, are stained by Gram and are useful for identification and rapid diagnosis. Particle agglutination tests are also useful for antigen detection of Haemophilus influenzae.
Patients with systemic Haemophilus influenzae infections require prompt antimicrobial therapy. Serious infections are treated with broad-spectrum cephalosporins. Less severe infections can be treated with ampicillin or fluoroquinolones.
The primary approach to preventing Haemophilus infections is active immunisation with purified capsular PRP.

13. Staphylococcus aureus



Staphylococcus aureus belongs to gram-positive cocci. It has a spherical shape, with no catalase activity and incapable of producing endospores. The genus Staphylococcus refers to the fact that they can grow in shapes of grapes.
Staphylococci are nonmotile, facultatively anaerobic, need a high concentration of salt in the media they grow and a temperature between 18^{o}C and 40 ^{o}C.
The outermost layer of staphylococci is covered with a polysaccharide capsule. Staphylococcus aureus has 11 capsular serotypes, with serotypes 5 and 7 most commonly causing infections. A slime layer consisting of monosaccharides, proteins and small peptides is produced by most staphylococci and is done so the bacteria can attach them selves to tissues.
Half of the cell wall weight is associated with the peptidoglycan, a feature common to gram-positive bacteria. The enzymes that catalyse the construction of the peptidoglycan layer are called penicillin-binding proteins and are the targets of penicillins.
Teichoic acid is another major component of the cell wall of staphylococci, is species-specific and is bound covalently to the peptidoglycan layer.
The surface of most Staphylococcus aureus strains is coated with protein A. This protein is bound to the peptidoglycan layer and has a unique affinity to binding the Fc receptor of IgG. Additionally, protein A is used as an identification marker for Staphylococcus aureus.
An important virulence factor of Staphylococcus aureus is the presence of coagulase (clumping factor) on its surface. This protein binds fibrinogen and converts it into insoluble fibrin, causing staphylococci to clump or aggregate.
Staphylococcus aureus produces many toxins, including five cytolytic toxins (alpha, beta, delta, gamma and Panton-Valentine leukocidin), two exfoliative toxins (A and B), eight enterotoxins (A to E, G to I) and toxin shock syndrome toxin-1 (TSST-1). The last three are considered also as superantigens.
Staphylococcus aureus causes disease through the production of toxin or through the direct invasion and destruction of tissue.
Toxin-mediated disease include scaled-skin syndrome, food poisoning and toxic shock. Suppurative infections include impetigo, folliculitis, furuncles, carbuncles, bacteremia, endocarditis, pneumonia, osteomyelitis and septic arthritis.
Staphylococcus aureus colonies will gradually turn yellow and can be also isolated on selective media such as mannitol-salt agar.

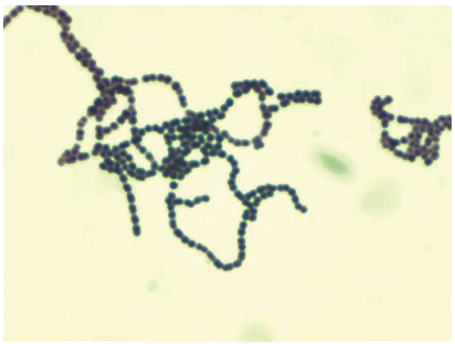
Staphylococci quickly develop drug resistance after penicillin was introduced and today, less than 10% of all strains are susceptible to this antibiotic. This resistance is mediated

by penicillinase production. Oral antibiotics used for outpatients include clindamycin, trimethoprim-sulfamethoxazole or doxycycline. Certain Staphylococcus aureus isolates have shown resistance to vancomycin.
Staphylococci are ubiquitous organisms present on the skin and mucous membranes of healthy people and can easily penetrate through skin cuts or wounds. However, the infectious dose (number of organisms required to cause an infection) is generally large unless a foreign body is present (dirt, splinter, stitches).

14. Coaggulase-negative staphylococci

15. Streptococcus, general characteristics and classification

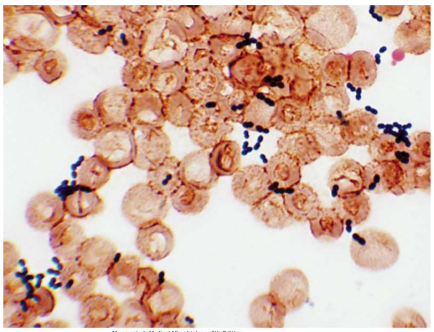
The genus Streptococcus is a diverse collection of gram-positive cocci typically arranged in pairs or chains. Most species are facultative anaerobes and some are capnophylic. Their nutritional requirements are complex, necessitating blood-enriched media. They ferment carbohydrates producing lactic acid and are catalase-negative.
Streptococci are spherical cocci arranged in short chains in clinical specimens or longer chains when grown in liquid media. Growth is optimal in blood-enriched media but inhibited when there is a high concentration of glucose. Colonies begin to form after 24 hours of culture and have a white colour with a degree of hemolysis, according to the species.



Numerous streptococci are pathogenic to humans and cause disease. They are classified according to different criteria. Serologic properties: Lancefield groupings (A to W), hemolytic patterns: complete hemolysis (β), incomplete hemolysis (α) and no hemolysis (γ) and biochemical (physiologic) properties.
Beta-hemolytic streptococcal strains and only a few alfa and gama-hemolytic strains possess group-specific antigens, most of which are cell wall carbohydrates. These antigens can be readily detected by immunologic assays. The rest of the alfa and gama-hemolytic strains are identified by biochemical tests.
The most medically-importantspecies of Streptococcus are Streptococcus pyogenes, Streptococcus agalactiae, Steptococcus pneumoniae and Streptococcus arginosus.

16. Enterococcus and viridans streptococci

Enterococci were previously classified as group D streptococci because they possess the D antigen on their cell wall. Relatively few species are important human pathogens and they are: Enterococcus faecalis, Enterococcus faecium, Enterococcus gallinarum and Enterococcus casseliflavus. The last two are vancomycin-resistant.
Enterococci are gram-positive cocci typically arranged in pairs and short chains. Under the microscope, they can't be differentiated from Streptococcus pneumoniae. The cocci grow both in anaerobic and aerobic conditions and in a broad temperature spectrum (10 to 45 ^{o}C). Their nutritional needs are complex and colonies appear 24 hours after incubation, with non-hemolytic, alfa-hemolytic and rare beta-hemolytic areas. The bacteria can grow in the presence of high NaCl concentration or bile salts.
Enterococci have no potent toxin or cell wall virulence factor. For this reason, they are typically considered to have limited potential for causing disease. They do possess, though, surface adhesin proteins that allow them to bind to the intestinal or vaginal epithelium in human hosts and secrete proteins with hemolytic and proteolytic activity. They can't avoid phagocytosis but can inherate resistance to certain antibiotics.



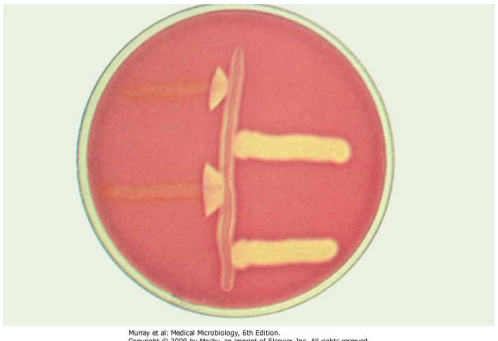
Despite the limited virulence of enterococci, they are capable of causing serious disease, especially in hospitalised patients. The urinary tract, peritoneum and heart tissue are sites most commonly infected by enterococci. Patients with urinary or intravascular catheters and patients receiving broad-spectrum antibiotics are particularly prown to enterococcal infection. A particularly severe complication of enterococcal bacteremia is endocarditis, a disease with a high mortality rate.
Enterococci grow readily on nonselective media such as blood or chocolate agar. Antimicrobial therapy for enterococcal infections is complicated because most antibiotics aren't bactericidal at clinically relevant concentrations. Traditionally, therapy consists of a combination of aminoglycosides and cell wall-active antibiotics (ampicillin or vancomycin). However, 25% of enterococci are resistant to aminoglycosides. Also, Enterococcus faecium is resistant to ampicillin and a majority of them to vancomycin. Newer antibiotics developed against enterococci include linezolid and fluoroquinolones. Prevention of enterococcal infections is difficult. Careful restriction of broad-spectrum antibiotic use reduces the risk of infection with enterococci.

The viridans group of streptococci is a heterogenous collection of alfa-hemolytic and nonhemolytic streptococci. Their name is derived from their ability to produce a green pigment on blood agar media. The most important species of the streptococcus viridans

group are Streptococcus anginosus and Streptococcus pneumoniae. Viridans streptococci colonise the oropharynx, GIT and genitourinary tract. They don't colonise the skin because of fatty acids present on it, that are toxic for the bacteria. Like other streptococci, viridans streptococci require complex nutrional media such as blood-enriched agar and a 5% to 10% carbon dioxide augmented atmosphere.

17. Beta-hemolytic streptococci

This group of gram-positive rods consists of two medically important species, Streptococcus pyogenes and Streptococcus agalactiae.
Streptococcus pyogenes is arranged in short chains. It grows on blood-enriched agar media and usually needs about 24 hours of incubation. The colonies that appear are white and thin, with large zones of beta-hemolysis.
Within the cell wall are group-specific and type-specific antigens. The group-specific carbohydrate is the Lancefield group A antigen. M protein is the major type-specific protein associated with virulent strains. Other important components of the cell wall include M-like proteins, lipoteichoic acid and F protein. Some strains of Streptococcus pyogenes form an outer hyaluronic acid capsule, making it indistiguishable from mammalian hyaluronic acid in connective tissue.
The virulence of group A streptococci is the ability to avoid opsonisation and phagocytosis, adhere to the surface of host cells, invade the epithelium and produce a variety of toxins and enzymes.
Diseases caused by Streptococcus pyogenes include pharyngitis, scarlet fever, pyoderma, cellulitis, necrotising fasciitis, streptococcal toxic shock syndrome, rheumatic and acute glomerulonephritis.
Streptococcus pyogenes is very sensitive to penicillin. An oral cephalosporin can be used in patients with beta-lactam intolorance. However, this treatment is ineffective in patients with mixed infection with Staphylococcus aureus. In this case, a combination of vancomycin and oxacillin is used. Patients with chronic rheumatic fever need long-term antibiotic prophylaxis to prevent recurrence of the disease.
Streptococcus agalactiae also forms short chains, making it indistinguishable from Streptococcus pyogenes. It also grows well in nutritionally-rich media and in contrast to the pyogenes species, agalactiae colonies are larger and have a narrower beta-hemolytic zone.

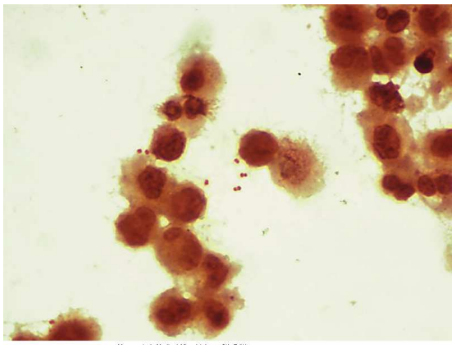


The most important virulence factor of Streptococcus agalactiae is the polysaccharide capsule. It interferes with phagocytosis until the patient develops type-specific

antibodies. Sialic acid inhibits activation of the alternative pathway of complement by interfering with phagocytosis.
Disease caused by Streptococcus agalactiae is divided into early-onset neonatal, late-onset neonatal, infections in pregnant women and infections in men and nonpregnant women.
Group B streptococci are identified in the laboratory primarily by a negative catalase test, a positive CAMP test and hydrolysis of hippurate. They are definitely identified by group-specific carbohydrates.
Group B streptococci are susceptible to penicillin, which is the drug of choice. However the MIC is 10 times that of Streptococcus pyogenes. Also, some tolerance to penicillin has been observed so a combination of penicillin with aminoglycosides is frequently used. Vancomycin is an alternative therapy for patients allergic to penicillin.
Chemoprophylaxis should be used for all women who are either colonised or at high risk. Intravenous penicillin administered 4 hours before delivery is recommended. Penicillin-allergic women are given vancomycin or cefazolin.

18. Immunopathological sequels of streptococcal infection

19. Neisseria



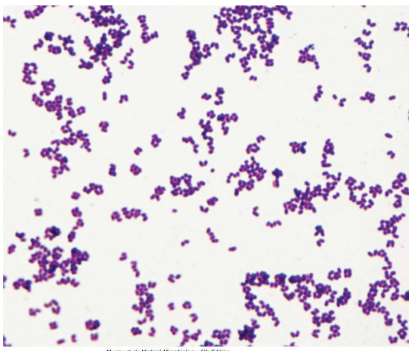
The genus Neisseria consists of 10 species found in humans with two, Neisseria gonorrhoeae and Neisseria meningitidis, strictly human pathogens.
Neisseria gonorrhoeae is always considered a pathogen and is the causative agent of the most common sexually transmitted disease, gonorrhea. Neisseria meningitidis is a paradox. This bacterium colonises the oropharynx of healthy people but is also the second causative agent of human meningitis.
Neisseria species are aerobic, gram-negative bacteria, typically coccoid shaped and arranged in pairs with adjacent sides flattened. They are nonmotile and don’t form endospores. All species are oxidase-positive and most are catalase-positive. Neisseria gonorrhoeae produces acid by oxidising glucose and Neisseria meningitidis does so by oxidising glucose and maltose.

Neisseria meningitidis has variable growth on nutrient agar and Neisseria gonorrhoeae is a fastidious organism requiring complex media for growth (all species require cysteine and an energy source such as glucose). The best medium for culture of both bacteria is

chocolate agar and at a temperature of 35 to 37 ^{o}C. Neisseria gonorrhoeae requires a humid atmosphere with 5% carbon dioxide.
The structure of Neisseria is that of a typical gram-negative bacterium. It has a thin peptidoglycan layer between the inner membrane and outer membrane. The major virulence factor of Neisseria meningitidis is the polysaccharide capsule. Both pathogenic and non-pathogenic strains of Neisseria have pili that extend from the cytoplasmic membrane through the outer membrane. They mediate attachment to host cells, transfer of genetic material and motility.
Another major antigen in the cell wall is lipooligosaccharide. This antigen is composed of lipid A and a core oligosaccharide but lacks the O-antigen polysaccharide found in lipopolysaccharide in most gram-negative rods. The lipid A acts as an endotoxin.
Gonococci attach to mucosal cells, penetrate into them and multiply and then pass through the cells into the subepithelial space where infection is established. The gonococcal lipooligosaccharide stimulates release of TNF-a causing local inflammation.
Like gonococci, meningococci are internalised into phagocytic vacuoles and are able to avoid intracellular death, replicate and then migrate to the subepithelial spaces.
Genital infection with Neisseria gonorrhoeae in men is primarily restricted to the urethra. A purulent urethral discharge and dysuria develop. 95% of all infected men have acute symptoms. The primary site of infection in women is the cervix. The organism can't infect the squamous epithelium of the vagina of postpubescent women.
Neisseria gonorrhoeae can be readily isolated from genital specimens if care is taken. Because other organisms normally colonise the mucosa of the genitals and rectum, both non-selective (chocolate agar) and selective media should be used to suppress their growth.
Neisseria meningitidis is generally present in large numbers in CSF, blood and sputum. Processing of these specimens should be done so with care because of high virulence.
Penicillin used to be the drug of choice in treating gonorrhea but today the MBC has risen and resistance has developed, along with a large population being allergic to beta-lactam antibiotics. Neisseria gonorrhoeae is also resistant to tetracyclines, erythromycin and aminoglycosides. Treatment includes administration of ceftriazone (if fluoroquinolones can't be used) and if infection with Chlamydia trachomatis hasn't been excluded, treatment should be combined with a single dose of azithromycin or a 1-week course of doxycycline.
Neisseria meningitidis remains susceptible to penicillin although certain strains have a low resistance. For patients with allergy to penicillin, broad-spectrum cephalosporines (ceftriaxone) or chloramphenicol can be used.
There are no preventive vaccines against Neisseria gonorrhoeae but prophylactive vaccines against the group-specific capsular polysaccharides of Neisseria meningitidis have been developed.

20. Corynebacterium diphtheriae and other corynebacteria. Arcanobacterium, Propionibacterium

The genus Corynebacterium belongs to aerobic gram-positive rods. It is a collection of 100 species and subspecies that have a cell wall with arabinose, galactose, meso-diaminopimelic acid and a short-chain mycolic acid.

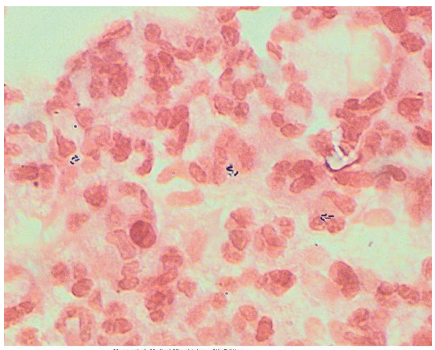


Corynebacteria are ubiquitous in animals and in plants and normally colonise the skin, upper respiratory tract, gastrointestinal tract and urogenital tract in humans. Almost all species of corynebacteria are oppotrunistic pathogens with only a few causing disease in humans. The most medically important species is Corynebacterium diphtheriae, the causative agent of diphtheria.
Corynebacterium diphtheriae is an irregularly staining, pleomorphic rod. Metachromatic granules have been observed in rods stained with methylene blue. Incubation usually takes one night and after that, large 1 to 3 mm colonies appear on blood agar.
The diphtheria toxin is the major virulence factor of Corynebacterium diphtheriae. This toxin is produced at the site of infection and then travels through the blood to cause systemic signs of diphtheria. The gene responsible for coding the diphtheria toxin is the tox gene, introduced to bacteria by a lysogenic bacteriophage (beta-phage).
Clinical presentation of diphtheria is determined by the site of infection, immune status of the patient and the virulence of the organism. According to the system affected, we distinguish respiratory diphtheria and cutaneous diphtheria.
The most important aspect of treatment for diphtheria is the early administration of diphtheria antitoxin to neutralise the exotoxin before it is bound by the host cell. Antibiotic therapy with penicillin or erythromycin is also used to eliminate Corynebacterium diphtheriae.
Symptomatic diphtheria can be prevented by actively immunising people with diphtheria toxoid. It's prepaired by formalin treatment of the toxin and is given to children in five doses with pertussis and tetanus antigens between the 2^{nd} and 18^{th} month of age and then 4^{th} and 6^{th} years.
Corynebacterium jeikeium is a well-recognised opportunistic pathogen in immunocompromised patients, particularly those with hematologic disorders or intravascular catheters. Most strains are very resistant to antibiotics.

Corynebacterium pseudotuberculosis is closely related to Corynebacterium diphtheriae and can carry the diphtheria gene although human disease is rarely observed.
Arcanobacterium is one of the more common coryneform genera associated with human

disease. This bacterium causes pharyngitis with a scarlet fever-like rash that resembles streptococcal disease with less common systemic complications such as septicemia and endocarditis. Infections are treated with penicillin or erythromycin.
Propionibacterium is another coryneform bacterium known for its unique metabolism. It produces propionic acid using special enzymes. It usually colonises the skin around sweat glands and sebaceous glands. They are usually ubiquitous and rarely cause disease in humans, with some cases of acne being reported. They live as intracellular parasites.

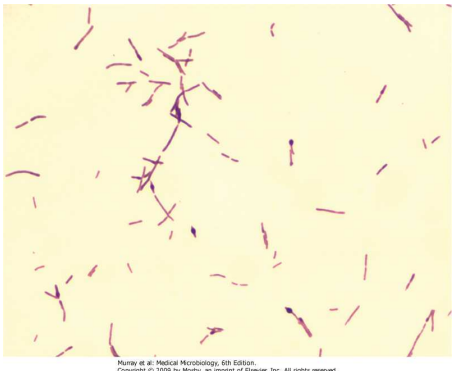
21. Erysipelothrix, Listeria



Listeria and Erysipelothrix belong to aerobic, non-spore forming, gram-positive rods. They are uniform is shape, unlike the irregular shape of Corynebacteria. They contain long-chaing mycolic acids in their cell walls making them difficult to stain by Gram technique so acid-fast staining was developed.
The genus Listeria consists of six species, with Listeria monocytogenes and Listeria ivanovii the only recognised pathogens. The former is a significant human pathogen whereas the latter is primarily an animal pathogen. Listeria monocytogenes is a short, non-branching, gram-positive, facultatively anaerobic rod capable of growth at a broad temperature range (1 to 45 ^{o}C) and in high concentration of salt. The short rods are arranged in singles, pairs or short chains and can be mistaken for Streptococcus pneumoniae or Enterococcus. The organism is motile at room temperature but less at 37 ^{o}C and have a characteristic movement. Listeria monocytogenes exhibits weak beta hemolysis when grown on sheep blood agar. Human disease is limited to certain groups such as neonates, elderly, pregnant women and immunocompromised patients.
Listeria monocytogenes is a facultative intracellular pathogen. After ingestion of contaminated food, Listeria monocytogenes is able to survive exposure to proteolytic enzymes, stomach acid and bile salts through the protective action of stress-response genes. They adhere to host cells through interactions of certain proteins on the surface of the bacteria with glycoproteins on the human cells. Acidic pH in Peyer’s patches activates the exotoxin listeriolysin O and two phospholipase C enzymes leading to release of the bacteria from the phagolysosome. They can survive in macrophages thus avoiding clearance by humoral immunity.

Two forms of neonatal disease have been described: early-onset disease (acquired transplacentally in utero) and late-onset disease (acquired at birth or soon after). Early-onset disease leads to abortion, stillbirth or premature birth. Granulomatosis

infantiseptica is a severe form of listeriosis, including multi-organ failure and a high mortality rate. Late-onset disease occurs 2 to 3 weeks after birth in the form of meningitis or meningoencephalitis with septicemia. Symptoms are non-specific and must be differentiated from streptococcal disease.
Most listeria infections in healthy adults are asymptomatic or in the form of a mild influenza-like illness. In contrast, illness in elderly people of immunocompromised patients is more severe.
Meningitis is the most common form of listeria infection in adults.
Gram-staining of CSF wont reveile bacteria because they are in a very low concentration. If detected, they appear as intra or extracellular, gram-positive coccobacilli.
Listeria grows on most common media with small, round colonies after a 1 to 2 day incubation period. Selective media and cold enrichment are necessary to detect listeriae in specimens.
Most antibiotics are bacteriostatic with Listeria monocytogenes and so the combination of gentamicin with either penicillin or ampicillin is the treatment of choice. Listeriae are naturally resistant to cephalosporins and resistance to macrolides and tetracyclines has been observed. Trimethoprim-sulfamethoxazole is bactericidal to Listeria monocytogenes and has been used successfully.
Prevention and control are difficult because listeriae are ubiquitous and most infections are sporadic. People should avoid eating raw or partially cooked animal foods and unwashed raw vegetables. There is no vaccine against Listeria monocytogenes.

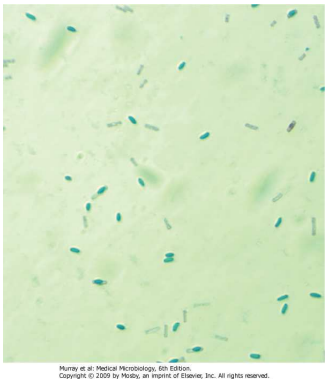


The genus Erysipelothrix contains three species, of which Erysipelothrix rhusiopathiae is responsible for human disease. It’s a gram-positive, non-spore forming rod that is distributed world wide in animals. The rods are slender and have a tendancy to form filaments. They are microaerophilic, prefering a reduced oxygen atmosphere and supplemented carbon dioxide.
Production of neuroaminidase is believed to be important for attachment and penetration into epithelial cells and a polysaccharide-like capsule protects the bacteria from phagocytosis.

Two primary forms of human disease have been recognised: a localised skin infection (erysipeloid) and a septicemic form. The systemic form of Erysipelothrix infections is uncommon but when manifested, it’s associated with endocarditis.

The rods are located only in the deep tissue of the lesion thus full-thickness biopsies should be obtained. Erysipelothrix rhusiopathiae isn't fastidious and grows on most conventional media. Growth is slow and cultures must be incubated for 3 days or longer before considered negative. The absence of motility and catalase distinguishes them from Listeria.
Erysipelothrix is susceptible to penicillin, which is the antibiotic of choice for both localised and systemic diseases. Cephalosporins, cabrapenems, macrolides, fluoroquinolones and clindamycin are also active in vitro although the organisms have variable susceptibility to sulfonamides and aminoglycosides and are resistant to vancomycin.

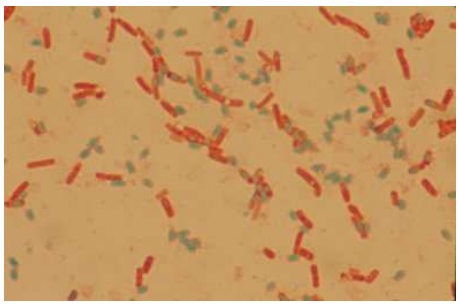
22. Bacillus



Bacillus belongs to the family Bacillaceae along with the genus Clostridium. There are almost 200 species of Bacillus but the medically most important are Bacillus anthracis and Bacillus cereus.
Bacillus anthracis is a large organism arranged as single or paired rods. Spores are observed in 2 to 3 day-old cultures but not in clinical specimens. It's an aerobic or facultatively anaerobic, gram-positive rod.
Virulent Bacillus anthracis carries genes for three toxin protein components: protective antigen, edema factor and lethal factor. They are non-toxic individually but when combined, produced highly toxic compounds. Protective antigen and edema factor produce the edema toxin and protective antigen and lethal factor produce the lethal toxin. A second important virulence factor carried by Bacillus anthracis is a prominent polypeptide capsule (poly-glutamic acid). The capsule is observed in clinical specimens but not in vitro unless specific growth conditions are used.
These two toxins (edema and lethal) and the capsule are the major virulence factors of Bacillus anthracis. The capsule inhibits phagocytosis of replicating cells. The edema toxin is responsible for fluid accumulation. Lethal toxin has a protease action over macrophages to release TNF-a, IL-1 and other proinflammatory cytokines. Protective antigen is the most immunogenic.

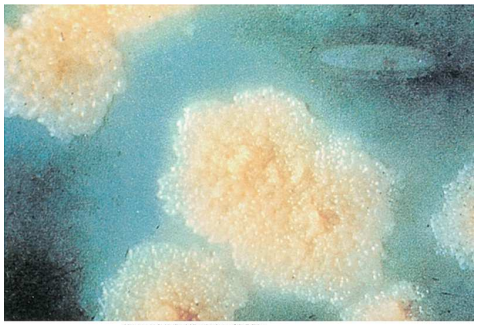
Bacillus anthracis causes cutaneous, gastrointestinal or inhalation anthrax.
Peripheral blood specimens can be stained by Gram and Bacillus anthracis appear as gram-positive rods. Colonies are not hemolytic (gama-hemolysis) in contrast to Bacillus

cereus, a characteristic used to distinguish the two. Bacillus anthracis appears nonmotile. Bacillus anthracis is susceptible to penicillin, doxycycline and ciprofloxacin but resistant to sulfonamides and extended spectrum cephalosporines. Because genes encoding resistance to penicillin and doxycycline have been transferred to Bacillus anthracis, recommended treatment includes a combination of either ciprofloxacin or doxycycline with another antibiotic such as rifampin, vancomycin or clindamycin.
Vaccination has been used to protect people who live in areas where anthrax is an endemic, people who work with animal products imported from countries with endemic anthrax and military personel.
Bacillus cereus is the most important opportunistic pathogen of the Bacillus species. Gastroenteritis caused by Bacillus cereus is mediated by one of two enterotoxins: heat-stable proteolysis-resistant enterotoxin (causes emetic form of the disease) and heat-labile enterotoxin (causes diarrheal form of the disease). The latter is similar to the enterotoxins produced by Escherichia coli and Vibrio cholerae.
Three toxins have been implicated in ocular infections by Bacillus cereus: necrotic toxin, cereolysin and phospholipase C.



Bacillus cereus is responsible for gastroenteritis, ocular infections and severe pulmonary disease.
Bacillus cereus can be cultured readily from specimens, except stool from patients with the emetic form. For confirmation, the implicated contaminated food should be cultured. Gastrointestinal forms of Bacillus cereus infections are short and usually need no antibiotic treatment. For other forms of the disease, the drugs used are vancomycin, clindamycin, ciprofloxacin and gentamicin. Penicillins and cephalosporines are ineffective. Eye infections should be treated rapidly.

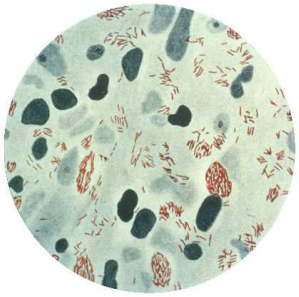
23. Mycobacterium tuberculosis, Mycobacterium bovis



Mycobacterium tuberculosis belongs to the family Mycobacterium, a nonmotile, non-spore-forming, aerobic family of rods. They are acid-fast bacteria, meaning they don't stain by Gram due to their highly lipid cell wall. They are resistant to many disinfectants and grow very slowly, usually 8 to 12 weeks.
The basic structure of the cell wall is typical of gram-positive bacteria: an inner plasma membrane, a thick peptidoglycan layer after it and no outer membrane. However, mycobacteria have anchored to their plasma membrane phosphatidylinositol manosides and lipoarabinomannan (LAM). LAM functions as an O-antigenic LPS.
Colonies are nonpigmented or have a light tan colour. Pigmented mycobacteria produce yellow carotenoids which may be stimulated by exposure to light.
Mycobacterium tuberculosis is an intracellular pathogen that is able to establish lifelong infection. At the time of infection, Mycobacterium tuberculosis enters the respiratory airways and minute infectious particles penetrate to the alveoli, where they are phagocytosed by alvelolar macrophages. It prevents formation of the phagolysosome by blocking a specific bridging molecule but the vacuole can style fuse with others containing nutrients, helping intracellular growth and replication.
Most immunocompetent patients infected with Mycobacterium tuberculosis involve pulmonary diseases (tuberculosis).
Diagnosis is based on: radiographic evidence of pulmonary disease, positive skin test reacticity and laboratory detection of mycobacteria. There may be no evidence of pulmonary disease in patients with disseminated tuberculosis.
The traditional test patients are submitted to is the tuberculin skin test. Reactivity to an intradermal injection of mycobacterial antigens can differentiate between infected and noninfected people.
In the laboratory, mycobacteria stain according to Ziehl-Neelsen method, as do acid-fast bacteria. This method uses decolouration with acid-alcohol solution and is then counterstained. Respiratory specimens are collected in 3 consecutive days. Sputum and other complicated specimens are first treated with a decontaminating reagent to remove other organisms that would confound results.
Treatment is difficult since slow growing mycobacteria are resistant to most antibiotics. Most treatments begin with 2 months isoniazid, ethambutol, pyrazinamide and rifampin followed by 4 to 6 months of isoniazid and rifampin or alternative combination drugs.
Vaccination with attenuated Mycobacterium bovis is commonly used in countries where tuberculosis is an endemic and responsible for significant mortality. Best candidates are young children. Unfortunately, immunocompromised patients can't be vaccinated.

24. Agents causing mycobacterioses, Mycobacterium leprae

Mycobacterium leprae belongs to the family of Mycobacteria and is the causative agent of leprosy (also known Hansen disease). They are nonmotile, non-spore-forming, acid-fast rods.
The basic structure of the cell wall is typical of gram-positive bacteria: an inner plasma membrane, a thick peptidoglycan layer after it and no outer membrane. However, mycobacteria have anchored to their plasma membrane phosphatidylinositol manosides and lipoarabinomannan (LAM). LAM functions as an O-antigenic LPS.
Colonies are nonpigmented or have a light tan colour. Pigmented mycobacteria produce yellow carotenoids which may be stimulated by exposure to light.
Because the bacteria grow very slowly, the incubation period is prolonged, with symptoms developing as long as 20 years after infection. As with Mycobacterium tuberculosis, clinical symptoms of leprosy depend on the immune status of the patient. Clinical presentation of leprosy varies between a tuberculoid form and a lepromatous form. Tuberculoid leprosy is characteristic with a strong immune system. Lepromatous leprosy is dealt with a strong antibody response but a specific defect in the cellular response to Mycobacterium leprae antigens. Thus, an abundance of bacteria are observed.
Leprosy is a chronic infection that affects the skin and peripheral nerves. The tuberculoid form is milder. The lepromatous form is associated with disfiguring skin lessions and involves the nasal mucosa.
Reactivity to lepromin, which is prepared from inactivated Mycobacterium leprae, is valuable for confirming the clinical diagnosis of tuberculoid leprosy. Papular induration develop 3 to 4 days after the intradermal injection. This test is ineffective in identifying patients with lepromatous leprosy.

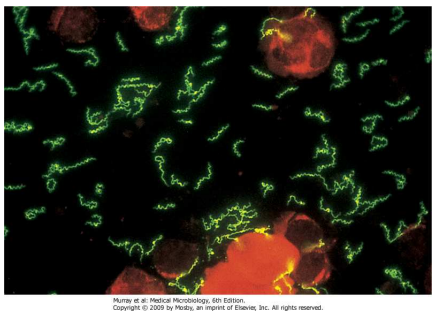


Culture, as with most mycobacteria, takes much more time than usual bacteria, ranging from 8 to 12 weeks. Contaminated specimens should be treated with a decontaminating agent to remove any other organisms that could interfere with the culture.
In the last decade, treatment of leprosy has successfully reduced the overall incidence of disease. The paucibacillary form should be treated with rifampicin and dapsone for a minimum of 6 months, whereas multibacillary form should have clofazimine added to the regimen and treatment should be extended to 12 months. Single-drug treatment shouldn’t be used for either form.

25. Collection of biological specimens and laboratory diagnostics of tuberculosis

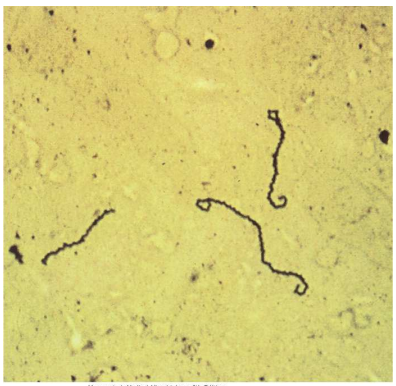
Mycobacteria that cause pulmonary disease (Mycobacterium tuberculosis), particularly in patients with cavitation (tissue destruction associated with hemoptysis), are abundant in the respiratory secretions. Recovery of the organisms is assured in patients from whom early morning respiratory specimens are collected for 3 consecutive days.
The in vitro growth of mycobacteria is complicated by the fact that most isolates grow slowly and can be obscured by the rapidly growing bacteria that normally colonise people. Thus specimens such as sputum are initially treated with a decontaminating agent (2% sodium hydroxide) to remove organisms that could confound results. Mycobacteria can resist weak alkali treatment, which kills most rapidly growing bacteria.
Formely, when specimens were inoculated onto egg-based and agar-based media, it generally took a long time for Mycobacterium tuberculosis to be detected. However, this time has been shortened through use of special broth cultures. Thus the average time for mycobacteria to grow has been decreased from 3 to 4 weeks to 10 to 14 days.
The traditional test to assess the patient's response to exposure to Mycobacterium tuberculosis is the tuberculin skin test. Reactivity to an intradermal injection of mycobacterial antigens can differentiate between infected and noninfected, with positive reactions developing 3 to 4 weeks after exposure to the organism. The only evidence of infection with mycobacteria in most patients is a lifelong positive skin test reaction and radiographic evidence of calcification of granulomas in the lungs or other organs.
The microscopic detection of acid-fast bacteria in clinical specimens is the most rapid way to confirm mycobacterial disease. The clinical specimen is stained by carbolfuchsin (Ziehl-Neelsen method) or fluorescent auramine-rhodamine dyes (fluorochrome method), decolorised with an acid-alcohol solution and then counterstained. The specificity of the test is greater than 95% when it is performed carefully.
Growth properties and colonial morphology can be used for the preliminary identification of Mycobacterium tuberculosis. However, species-specific molecular probes are the most useful means of identifying commonly isolated mycobacteria.

26. Treponema



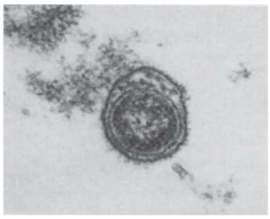
The genus Treponema belongs to the family Spirochaetales, a thin, helical, gram-negative family of bacteria. The most important species of the Treponema genus are Treponema pallidum and Treponema carateum. Treponema pallidum is the causative agent of syphilis.
Treponema pallidum is a thin, tightly coiled spriochete, with pointed straight ends. Three periplasmic flagella are inserted at each end. It is very difficult to grow Treponema pallidum on common laboratory media because the organism is highly dependent on host cell purines, pyrimidines and most amino acids. Also, like most spirochetes, Treponema is microaerophilic or anaerobic and extremely sensitive to oxygen toxicity.
The spirochetes are too thin to be seen with light microscopes in specimens stained with Giemsa or Gram. For this reason, darkfield illumination is used or staining with specific antitreponemal fluorescent antibodies.
The lack of species-specific antigens allows the spirochetes to evade the immune system. The bacteria can also avoid phagocytosis and adhere to host fibronectin, allowing direct interaction with the host tissues. It is believed that the tissue destruction associated with syphilis is due to the patient’s immune system respronce to infection.
In utero infections can lead to serious fetal disease, resulting in latent infections, multiorgan malformations or death of the fetus. Most infected infants are born without clinical evidence of disease, but rhinitis then develops and is followed by a widespread desquamating rash. Teeth and bone malformation, blindness and deafness are common in untreated infants who survive the initial phase.
Penicillin is the drug of choice for treating Treponema pallidum infections. Long-acting benzathine penicillin is used for early stages of infection and penicillin G is recommended for congenital and late syphilis. Tetracycline and doxycycline can be used as alternative antibiotics for patients with penicillin allergies. Only penicillin can be used for treatment of neurosyphilis, thus penicillin-allergic patients with neurosyphilis must undergo desensitization. Erythromycin and other macrolides are ineffective.
There is no vaccine against syphilis but practice of safe-sex techniques can limit possibility of infection.

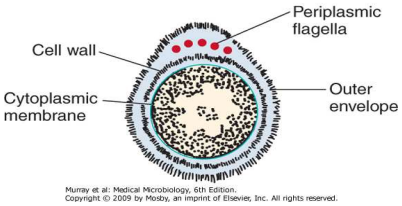
27. Leptospira



Leptospira, the second member of the Spirochaetales family, are subdivided into pathogenic and nonpathogenic.
Leptospires are thin, coiled spirochetes with a hook at one or both pointed ends. Motility is by means of two periplasmic flagella extending the length of the bacteria. They are obligate aerobes with optimum growth in vitamin-supplemented media.
Pathogenic leptospires can cause subclinical infection, a mild influenza-like febrile illness, or sever systemic disease (Weil syndrome), with renal and hepatic failure, extensive vasculitis, myocarditis and death. The severity of the disease is influenced by the host's immune system, the number of infecting bacteria and the virulence of them. Because leptospires are thin and motile, they can easily penetrate mucous membranes or skin through small cuts or abrasions. They can spread through the blood to all tissues. Leptospira interrogans multiply rapidly and damage the endothelium of small blood vessels. Organisms can be found in blood and CSF early in the disease and in urine during the later stages. Clearance happens when humoral immunity develops.
Because leptospira are thin, neither Gram nor silver stain can make them visible under light microscopes. Darkfield microscopy is also relatively insensitive. Fluorescein-labeled antibody preparations are used but not available in most clinical laboratories. Leptospires can be cultured on specially formulated media. They grow slowly, requiring incubation at 28 ^{o}C to 30 ^{o}C for as long as 4 months even though most cultures are positive in 2 weeks. Several specimens should be collected from blood, urine and CSF. Patients should be treated either with intravenous penicillin or doxycycline. Doxycycline but not penicillin can be used to prevent the disease in people exposed to infected animals or water contaminated with urine. Prevention is accomplished by vaccination of livestock and pets.

28. Borrelia



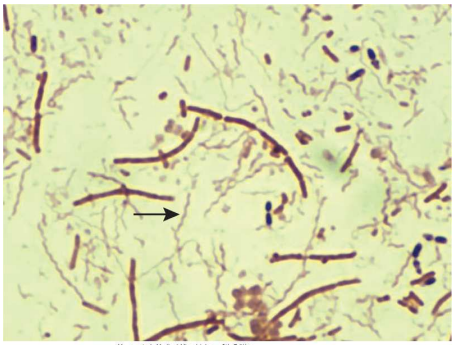


The genus Borrelia belongs to the family Spirochaetales which are weakly staining, gram-negative rods. Three species of Borrelia cause disease in humans and they are: Borrelia burgdorferi, Borrelia garinii and Borrelia afzelii. Borrelia genus are the causative agents of Lyme disease and relapsing fever.
Borrelia tend to be larger than other spirochetes, stain well with aniline dyes (e.g. Giemsa) and can be easily seen in light microscopy in smears of peripheral blood. Motility of the organism is due to the 7 to 20 periplasmic flagella present on it. Borreliae are microaerophilic and have complex nutritional requirements.
Culture is generally difficult so diagnosis is based on microscopy (relapsing fever) or serology (Lyme disease).
The growth of borreliae on both arthropod vectors and mammalian hosts is regulated by differential gene expression with up- or down-regulation of outer surface proteins. Spirochetes are not frequently isolated from specimens in late stages of the disease. The exact mechanism by which borreliae cause disease is unknown. Immune response to the organism is depressed at the time skin lesions initially develop but antibodies develop in a matter of months to years and are responsible for clearance of borreliae.
Nutritional requirements of Borreliae are complex and include N-acetylglucoseamine, glucose, long-chain fatty acids and amino acids. Duplication time is about 18 hours. Antibodies against borreliar antigens can be detected through immunofluorescence assays or enzyme immunoassays.
Relapsing fever has been treated most effectively with tetracyclines or penicillins. The drug of choice is tetracycline but is not recommended for pregnant women or young children. Vaccines are not available for relapsing fever.
The early manifestations of Lyme disease are managed with orally administered amoxicillin, doxycycline or cefuroxime. Patients with chronic symptoms should be treated symptomatically.

29. Campylobacter and Helicobacter

The genera Campylobacter and Helicobacter belong to the same family of spiral, gram-

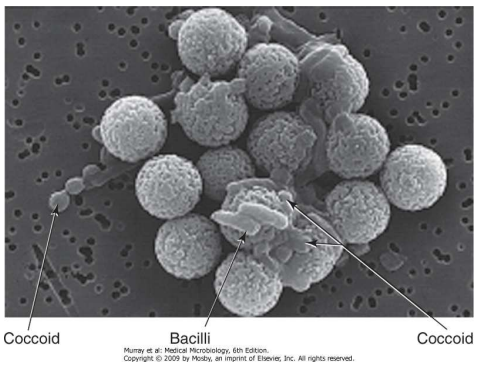
negative, nonfermentative, microaerophilic rods.



The genus Campylobacter consists of small, comma-shaped, gram-negative rods that are motile by means of polar flagella. Most species are microaerobic. Four species are associated with human disease: Campylobacter jejuni (most common cause of gastroenteritis), Campylobacter coli (2% to 5% of Campylobacter gastroenteritis), Campylobacter upsaliensis (uncommon cause of gastroenteritis) and Campylobacter fetus (common cause of bacteremia, septic thrombophlebitis, arthritis, septic abortion and meningitis).
Campylobacters have a typical, gram-negative cell wall structure. The major antigen is the lipopolysaccharide of the outer membrane. Campylobacter jejuni grows optimally at 42 ^{o}C. Their small size helps their recovery by filtration of stool specimens.
Although adhesins, cytotoxic enzymes and enterotoxins have been detected in this species, their specific role in disease remains unknown. The risk of disease is influenced by the infectious dose. The organisms are killed by gastric acid secretion and the immune status of the patient plays an important role.
Campylobacter jejuni gastrointestinal disease characteristically produces histologic damage to the mucosal surfaces of the jejunum, ileum and colon. The organisms invade through the epithelium into the lamina propria and cause local inflammation.
Campylobacters are thin and usually don’t stain easily with Gram. Despite this, observation of thin, S-shaped organisms in stool is very specific.
The selective media for culture of Campylobacter should contain blood or charcoal to remove toxic oxygen radicals amd antibiotics are added to inhibit growth of contaminating organisms. Campylobacters are slow growing and require 1 to 2 days.
Campylobacter gastroenteritis is typically a self-limited infection but antibiotics can be used in patients with severe septicemia. Campylobacters are susceptible to most macrolides (erythromycin, azithromycin), fluoroquinolones, clindamycin, amoxicillin, tetracyclines, aminoglycosides and chlroamphenicol.

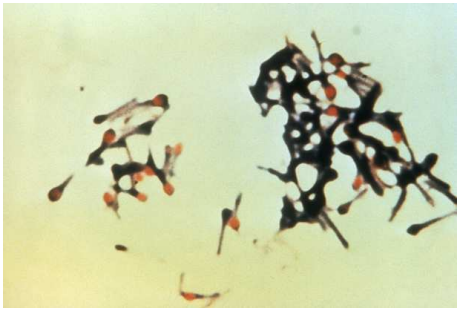
Helicobactes resemble Campylobacters in their appearance as spiral, gram-negative rods. The most important species, Helicobacter pylori, has been associated with gastritis, peptic ulcers, gastric adenocarcinoma and gastric MALT B-cell lymphomas.

Morphologically, they are similar to Campylobacters. Helicobacter pylori is highly motile via polar flagella and produce an abundance of urease. Most Helicobactes are catalase and oxidase-positive. Helicobacter lipid A has low endotoxin activity. Growth of Helicobacters requires a complex medium supplemented with blood, serum, charcoal, starch or ergg yolk. Microaerophilic conditions are required and a temperature between 30 ^{o}C and 37 ^{o}C is the optimal.
Initial colonisation of the stomach pylorus by Helicobacter pylori is accomplished by blockage of gastric acid productiong through bacterial acid-inhibitory proteins and neutralisation of gastric acids by the ammonia produced by bacteria urease. Localised tissue damage is mediated by urease byproducts mucinase and phospholipase.



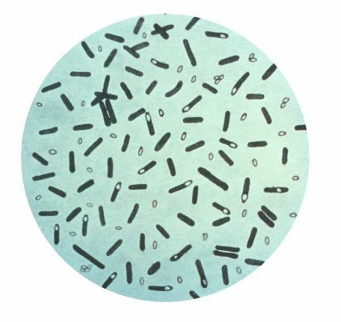
Helicobacter species are subdivided into gastric helicobacters and enterohepatic helicobacters.
Colonisation with Helicobacter pylori leads to histological evidence of gastritis. Helicobacter cinaedi and Helicobacter fenneliae can cause gastroenteritis and bacteremia most commonly in immunocompromised patients.
The greatest success in curing gastritis or peptic ulcer disease has been accomplished with the combination of a proton pump inhibitor (omeprazole), a macrolide (clarithromycin) and a beta-lactam (amoxicillin) with administration for 7 to 10 days initially.

30. Clostridium tetani



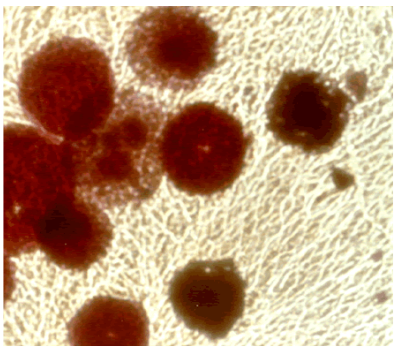
The species Clostridium tetani belongs to the family Clostridium, a group of anaerobic, spore-forming, gram-positive rods.
Clostridium tetani is a large, motile, spore-forming rod. The organism produces round, terminal spores that give it the appearance of a drumstick. Clostridium tetani is difficult to grow because it's extremely sensitive to oxygen toxicity and non-fermentative.
Although vegetative Clostridium tetani cells die rapidly due to exposure to oxygen, the spores formed allow the organism to survive the most adverse conditions. Also, it produces two toxins: tetanolysin (an oxygen-labile hemolysin) and tetanospasmin (a plasmid-encoded, heat-labile neurotoxin).
Tetanospasmin is produced during the stationary phase of growth, is released when the cell is lysed and is responsible for the clinical manifestations of tetanus. The intact toxin molecules are internalised in endosomal vesicles and transported in the neuron axon to motor neurons of the spinal cord. This leades to spastic paralysis.
The incubation period of tetanus varies from a few days to weeks. The incubation period is related directly to the distance of the infected wound from the CNS.
Generalised tetanus is the most common form. Involvement of the masseter muscles is the presenting sign in most patients.
Localised tetanus is when the disease remains confined to the musculature at the site of primary infection. A variant is cephalic tetanus, in which the primary site is the head.
Neonatal tetanus is typically associated with an initial infection of the umbilical stump that progresses to become generalised. The mortality exceeds 90%.
The diagnosis of tetanus is based on the clinical symptoms and not on microscopy. Culture of Clostridium tetani is usually unsuccessful and positive in only 30% of the time. Neither tetanus toxin or antibodies against the toxin are detectable in a patient. If the organism is recovered in culture, the toxin can be used in a test for neutralisation by antitoxin in mice.
Treatment of tetanus requires debridement of the wound, use of metronidazole, passive immunisation with human tetanus antibodies and vaccination with tetanus toxoid. Penicillin inhibits GABA activity and should not be used. Vaccination with tetanus toxoid happens in 3 doses, once every 10 years.

31. Clostridium botulinum



Clostridium botulinum, the etilogic agent of botulism, is a heterogenous group of large, fastidious, spore-forming, anaerobic rods. They are subdivided into 4 subspecies. There are seven Clostridium botulinum toxins (A to G) and human disease is associated to A, B, E and F.
Like tetanus toxin, Clostridium botulinum toxin consists of a small subunit with zinc-endopeptidase activity and a large subunit. In contrast with tetanus toxin, Clostridium botulinum toxin is complexed with nontoxic proteins that protect the neurotoxin during passage through the digestive tract. Also in contrast to the tetanus toxin, botulinum toxin remains at the neuromuscular junctions. The clinical result is flaccid paralysis. Recovery requires regeneration of the nerve endings.
Patients get foodborne botulism after consumption of contaminated food. Mortality has decreased from 70% to 5% to 10%.
Infant botulism is caused by a neurotoxin produced in vivo by Clostridium botulinum colonising the gastrointestinal tract of infants. In the absence of competitive bowel microbes, the organism can proliferate. Infacts affected are usually under 1 year of age.
Wound botulism develops from toxin production by Clostridium botulinum in contaminated wounds.
Isolation of Clostridium botulinum can be improved by heating the specimen at 80 ^{o}C for 10 minutes, killing nonclostridial cells. Culture happens on nutritionally enriched anaerobic media.
Treatment includes adequate ventilatory support, elimination of the organism from the GIT through gastric lavage and metronidazole or penicillin therapy and use of trivalent botulinum antitoxin versus toxins A, B and E.

32. Mycoplasma and Ureaplasma

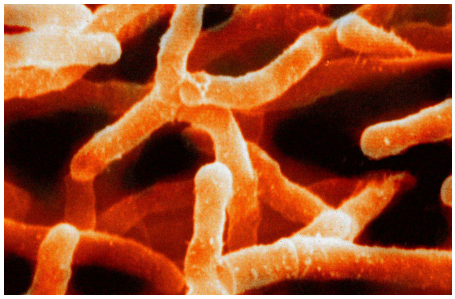


The genera Mycoplasma and Ureaplasma belong to the family Mycoplasmataceae, part of the class Mollicutes. The most medically important species are Mycoplasma pneumoniae and Ureaplasma urealyticum.
Mycoplasma and Ureaplasma organisms are the smallest free-living bacteria. They are unique since they do not have a cell wall and their cell membrane contains sterols. The absence of a cell wall makes these organisms resistant to antibiotics that target cell wall synthesis (e.g. penicillin, cephalosporin, vancomycin etc).
The mycoplasmas are pleomorphic, varrying in shape from coccoid to rods.
Mycoplasmas are facultatively anaerobic (except for Mycoplasma pneumoniae, a strict aerobe) and require exogenous sterols supplied by animal serum. They generally grow slowly and form colonies difficult to detect if not incubated for an extended period of time.
Because mycoplasmas don’t have a cell wall, the major antigenic determinants are membrane glycolipids and proteins.
Mycoplasma pneumoniae is an extracellular pathogen that adheres to respiratory epithelium by means of a specialised attachment structure formed at one end of the cell. It consists of a complex of adhesion proteins with P1 adhesin being the most important. Ciliostasis occurs, after which the cilia and then the epithelium are destroyed. This interferes with normal upper respiratory tract clearance, allowing the lower airways to be contaminated with microbes. Mycoplasma pneumoniae functions as a superantigen, stimulating inflammatory cells to migrate to the site of infection and release of cytokines
Exposure to Mycoplasma pneumoniae typically results in asymptomatic carriage. The most common clinical presentation is tracheobronchitis. Primary atypical pneumonia may also develop. Secondary complications include neurologic abdnormalities, pericarditis, hemolytic anemia, arthritis and mucocutaneous lesions.
Ureaplasma urealyticum can cause nongonococcal urethritis, pyelonephritis and spontaneous abortion or premature birth.
Microscopy is of no diagnostic value since Mycoplasmas stain poorly because of absence of cell wall. Antigen tests are also poorly sensitive and non-specific and aren’t recommended.
Unlike other Mycoplasmas, Mycoplasma pneumoniae is a strict aerobe. It can be isolated from throat and bronchial washings or expectorated sputum. Specimens should be inoculated onto media containing special supplements (sterols, yeast extract, glucose, a pH indicatior and penicillins). Duplication time is about 6 hours.

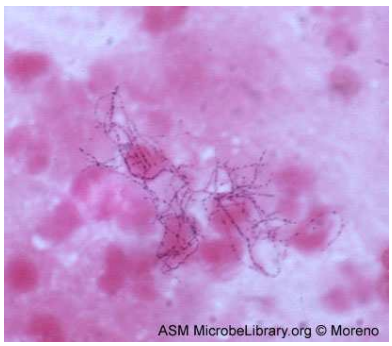
About 32% of cultures are positive within 2 weeks, with most requiring an extended period of time (6 weeks).

Tests for antibodies against Mycoplasma pneumoniae are available and include a serologic reference standard.
Erythromycin, tetracyclines and fluoroquinolones are equally effective in treating Mycoplasma pneumoniae. Erythromycin is used to treat Ureaplasma infections because the organisms are resistant against tetracyclines.
Prevention of infection with Mycoplasma pneumoniae includes isolation of infected individuals, since the disease spreads via close contact. Vaccines used in the past have been proven ineffective. Ureaplasma infection is transmitted sexually, therefore practice of safe-sex reduces the risk of infection.

33. Actinomyces and Nocardia

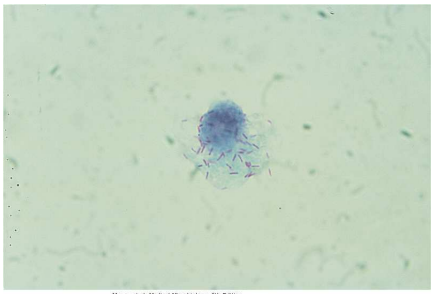


Actinomyces oganisms are facultatively anaerobic or strictly anaerobic, gram-positive rods. They aren’t acid-fast, in contrast to the morphologically similar Nocardia, they grow slowly and tent to cause chronic, slowly developing infections. They typically develop delicate filamentous forms or hyphae (resembling fungi) in cultures or clinical specimens. The most important species causing disease are Actinomyces israelii, Actinomyces naeslundii, Actinomyces radingae and Actinomyces turicensis.
Actinomyces colonise the upper respiratory tract, the GIT and the human genital tract. They have a low virulence potential and cause disease only when the normal mucosal barriers are disrupted.
Disease caused by Actinomyces is called actinomycosis. Infections nowadays are rare and usually involve polymicrobic, oral infections such as endodontic infections.
Most Actinomyces infections are of the cervicofacial type.
Abdominal actinomycosis can spread throughout the abdomen, potentially involving virtually every organ system. Pelvic actinomycosis can occur as a relatively benign form of vaginitis or more commonly there can be extensive tissue damage. In CNS actinomycosis a solitary brain abscess is formed.
Because the organisms are sparse in the contaminated tissue, a large amount of specimens should be obtained for laboratory diagnosis.
Growing of Actinomyces takes time (2 weeks or more). Colonies appear white and have a domed surface. After a week of incubating, the colonies look irregular and resemble the top of a molar tooth in shape.
Treatment involves the combination of drainage of the abscess or surgical debridement of the involved tissues and prolonged administration of penicillin, carbapenems, macrolides or clindamycin. Good oral hygiene lowers the risk of infection.

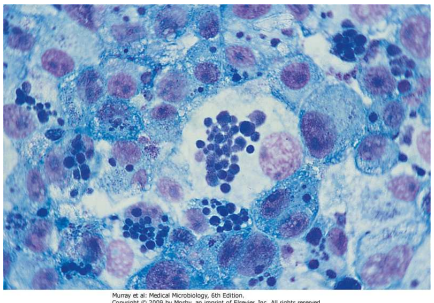


Nocardiae are strict aerobic rods that form branches filamentous form in tissues and culture. Unlike Actinomyces, Nocardia genera are weak acid-fast bacteria, meaning a weak decolorising HCl solution must be used to demonstrate the acid-fast property. The reason Nocardiae stain poorly with Gram (appear gram-negative with intracellular gram-positive beads) is that they have a cell wall similar to mycobacteria.
Nocardia is catalase-positive, oxidises carbohydrates and can grow on most nonselective laboratory media. Their growth is slow, usually requiring 3 to 5 days before colonies can be observed. The appearance is waxy and and the colour varies from white to orange.
The presence of aerial hyphae and acid-fastness are unique to Nocardia and can be used as a rapid identification test.
Nocardia causes bronchopulmonary disease in immunocompromised patients with a high predilection for hematogenous spread to the CNS or skin. Patients at greatest risk of infection are those with T-cell deficiencies. The most common causative agent of this is Nocardia brasiliensis.
The primary virulence factor of Nocardiae is the ability to avoid phagocytosis. During phagocytosis, oxidative burst occurs, releasing toxic oxygen metabolites. Nocardiae are protected by their catalase and superoxide dismutase enzymes.
Nocardiae can also replicate in macrophages. This happens by preventing phagolysosome formation.
Bronchopulmonary disease caused by Nocardia species can’t be distinguished from infections caused by other pyogenic ogranisms, although Nocardia infections tend to develop more slowly and mostly in immunocompromised patients.
Cutaneous infections may be primary or as a result of secondary spread from a primary infection.
Multiple sputum specimens should be collected from patients with pulmonary disease.
The ogranisms grow on media in an atmosphere of 5% to 10% carbon dioxide. Nocardia can also be used on media used for recovery of Legionella species (buffered chracoal yeast extract agar).
Nocardia infections are treated with the combination of antibiotics and appropriate surgical intervention. Trimethoprin-sulfamethoxazole is used to treat local infections. In more severe disease, amikacin and carbapenems are used in combination or broad-spectrum cephalosporin. Therapy should be extended over a period of 6 weeks or more.
Prevention can be accomplished by proper wound care.

34. Rickettsia, Orientia, Ehrlichia



Rickettsia genus belongs to the family of Rickettsiaceae, a group of intracellular, aerobic, gram-negative rods.
The cell wall of Rickettsia is typical for gram-negative bacteria, with a peptidoglycan layer and a lipopolysaccharide. They stain weakly by Gram and are best seen when stained by Giemsa. The bacteria have no flagella and are surrounded by a thin slime layer.
They enter eukaryotic cells by attachment to host cell surface receptors and stimulate phagocytosis. After engulfment, Rickettsia degrades the phagosome membrane by phospholipase activity.
There are 3 Rickettsia species that are most medically important: Rickettsia ricketssii, Rickettsia prowazekii and Rickettsia typhi.
Rickettsia ricketssii causes Rocky Mountain spotted fever. Disease isn't caused by release of a toxin but from proliferation of the bacteria in host cells.
The drug of choice is doxycycline and is even recommended for pregnant women and young children. Fluoroquionolones show good signs in in vitro testing but clinical experience proves otherwise.
Rickettsia prowazekii is the etiologic agent of epidemic or louse-borne typhus whereas Rickettsia typhi causes endemic or murine typhus.
Treatment includes administration of tetracyclines and a formaldehyde-inactivated typhus vaccine is available.
Orientia genus, specifically Orientia tsutsugamushi, is the etiologial agent of scrub typhus, a disease transmitted to humans by mites. Infection is also present in the rodent population.
Doxycycline is the drug of choice. No vaccine is available.
Ehrlichia, unlike Rickettsia and Orientia, belongs to the family of Anaplasmataceae. It's a group of obligate intracellular bacteria that survive in cytoplasmic vacuoles of mammalian hematopoietic cells.

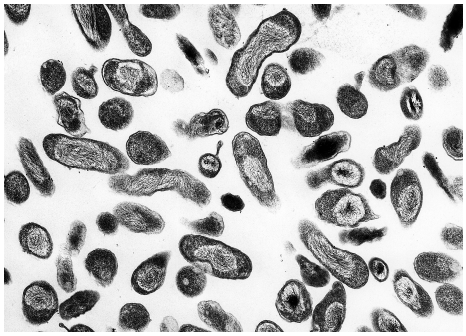


In contrast to Rickettsia and Orientia, Ehrlichia remains in the phagocytic vacuole after

entry into the host cell. Fusion with the lysosome is prevented by appropriate receptors. Progressive infection icludes lysis of infected cells, release of bacteria and subsequent infection of new cells. Ehrlichia can be detected in cells stained by Giemsa.
Cell wall structure is similar to gram-negative bacteria, however Ehrlichia lacks genes that code for peptidoglycan or lipopolysaccharide synthesis.
Their intracellular location protects the organisms from the host's antibody response.
Human Monocytic Ehrlichiosis is caused by Ehrlichia chaffeensis, following infection of monocytes and mononuclear phagocytes in tissues and organs.
Therapy includes administration of doxycycline but must be delayed until laboratory confirmation of the disease. Rifampin is used to treat patients unable to tolerate doxycycline. Resistance has been detected against fluoroquinolones. Penicillins, cephalosporines, macrolides and aminoglycosides are all ineffective.

35. Coxiella burnetii, Bartonella quintana

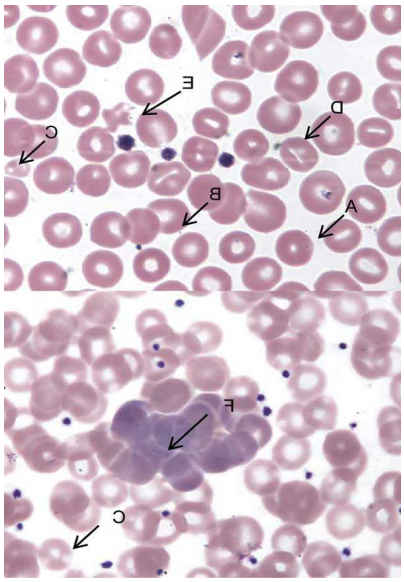
Coxiella burnetii was originally classified with Reckettsia because it stains weakly as gram-negative, it's an intracellular parasite of eukaryotic cells and associated with arthropods (ticks) but now is recognised to be related to Legionella. Coxiella burnetii is the causative agent of Q (query) fever.



There are two structural derivatives of Coxiella burnetii, small cell variants (extremely resistant to environmental stress) and large cell variants (multiply in host monocytes and macrophages). Exposure of Coxiella burnetii to the environment, SCV are phagocytosed, rearragned into LCV and phagolysosome formation follows. LCVs replicate in the acid environment of the phagolysosome. At some point, LCVs reorganise into SCVs, which are released into the tissues, remaining infectious for months to years.
Coxiella can undergo antigenic variation in expression of the cell wall LPS antigen.
Most infected patients are asymptomatic and most symptomatic infections are mild, present as a flu-like infection. Less than 5% of acutely infected individuals present severe disease with pneumonia, hepatitis or isolated fevers the most common presentations. Chronic Q fever develops months to years later, mostly in patients with underlying heart valvular problems or immunosuppression. Subacute endocarditis is the most common presentation but symptoms are nonspecific.

Q fever is currently diagnosed by culture, serology or PCR, with serology being the most common test. A diagnosis of chronic Q fever is confirmed by the demonstration of

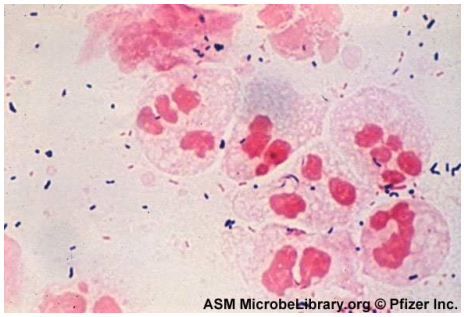
antibodies against both Phase I and II antigens, with Phase I titers being higher. Chronic infections are treated according to clinical experience. For acute infections, the drugs of choice are tetracyclines (doxycycline). Chronic infections should be treated with a prolonged administration of doxycycline and the alkalinising agent hydroxychloroquinine. Macrolides, aminoglycosides and beta-lactams are ineffective. Inactivated whole-cell derivatives and partly purified antigen vaccines for Q fever have been developed and vaccines from Phase I organisms have shown to be most effective.



Bartonella quintana, a member of the gram-negative rod genus Bartonella, is the causative agent of trench fever (5-day fever) meaning the fever recurs at 5-day intervals. Although non-fatal, the disease can be severe. Exposure to contaminated feces of human body louse spreads the disease from person to person.
Patients with immunodeficiencies, especially HIV, experience recurrent fevers with bacteremia and bacillary angiomatosis. Bacteremia may lead to endocarditis or, more commonly, vascular proliferative diseases of the skin, subcutaneous tissues or bone. Treatment of Bartonella involves a 4 – 6 week administration of doxycycline, erythromycin or azithromycin.

36. Chlamydia

The genus Chlamydia is a member of the Chlamydiaceae family, a group of obligate intracellular parasites, originially thought to be viruses. The most medically important species of this genus is Chlamydia trachomatis, the causative agent of chlamydia, a sexually transmitted disease.
Chlamydiaceae have the ability of forming metabolically inactive infectious forms (elementary bodies) and metabolically active noninfectious forms (reticulate bodies). Much like spores, elementary bodies are resistant to many harsh environmental factors. Although these bacteria lack the ridgid peptidoglycan layer (gram-negative bacteria), their central dense core is surrounded by a cytoplasmic membrane and a double-layer outer membrane. The cell wall contains LPS with weak endotoxin activity. The species-specific structure of Chlamydiaceae is their major outer membrane protein (MOMP).



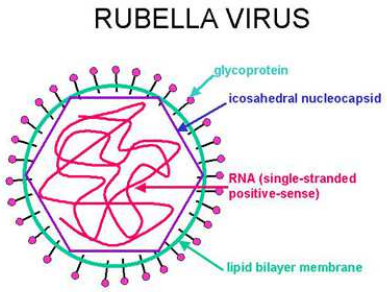
The Chlamydiaceae are energy parasites, because they use the host cell ATP for their energy requirements. It takes 48 to 72 hours from the time of infection for the infected cells to rupture and release the infectious bacteria.
Chlamydia trachomatis has a limited host range, with infections restricted to humans. The species responsible for disease are subdivided into two biovars: trachoma and LGV (lymphogranuloma venereum).
Cells that can be infected by elementary bodies of Chlamydia trachomatis are limited to nonciliated columnar, cuboidal and transitional epithelium cells, which are found on the mucous membrane of the urethra, endocervix, endometrium, fallopian tubes, anorectum, respiratory tract and conjuctiva.
Chlamydia gain access through abrasions or lacerations. In LGV, the lesions form in the lymph nodes, draining the site of primary infection. The lesions may become necrotic, attracking PMN leukocytes and cause the inflammatory process to spread to surround tissues. Rupture of the lymph nodes leads to severe inflammatory response.
Infection doesn’t confer long-lasting immunity. Reinfection characteristically induces a vigorous inflammatory reaction with subsequent tissue damage.
Chlamydia trachomatis manifests its self in many forms of disease: trachoma, adult inclusion conjuctivitis, neonatal conjuctivitis, infant pneumonia, ocular lymphogranuloma venerum, urogenital infections and lymphogranuloma venereum.

The first method used to detect Chlamydia trachomatis is Giemsa staining of cell scrapings. However the method is insensitive and not recommended.

There are two general approaches to detect chlamydial antigens: direct immunofluorescence with fluorescein-conjugated monoclonal antibodies and ELISA. In both methods, antibodies are directed against chlamydial MOMPs or cell wall LPS. Serologic tests for antibodies against Chlamydia trachomatis in urogenital infections is nonspecific because it can't differentiate between current and past infections.
Treatment of patients with lymphogranuloma venereum is a 3-week administration of doxycycline. Children younger than 9 years, pregnant women and doxycycline-intolerant patients should be treated with erythromycin. Ocular and genital infections are treated with a single dose of azithromycin or 7 days of doxycycline. Newborn conjuctivitis and pneumonia should be treated with erythromycin for 10 to 14 days.
Practice of safe-sex greatly reduces the risk of infection with Chlamydia trachomatis.

37. Rubella virus

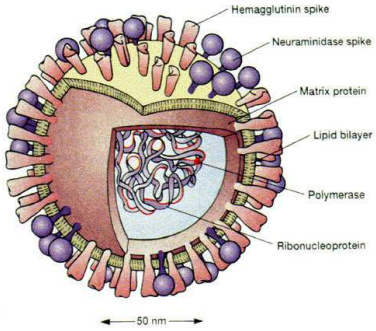
Rubella virus belongs to genus Rubivirus, a member of the family of Togaviridiae, a group of enveloped, positive, single-stranded RNA viruses.
Rubella virus, unlike other togaviruses, causes respiratory tract diseases and not readily detectable cytopathologic effects. It's one of five classic childhood exanthems and is the causative agent of German measles. Maternal rubella infection can lead to severe congenital defects in the infants.



Rubella infects the upper respiratory tract and then spreads to local lymph nodes, which leads to a period of lymphadenopathy. Viremia follows, spreading the virus throughout the body. Infection of other tissues and a characteristic mild rash result. This prodromal period lasts 2 weeks.
Antibodies are generated after the viremia and this correlates with the appearance of the rash (immune complexes). They contain spreading of the virus but cell-mediated immunity plays an important role in resolving the infection. Because only on serotype of rubella exists, one infection is enough for life-long immunity.
Disease in children is mild but in adults can be severe.
Isolation of the rubella virus is very difficult and detection can be done with RT-PCR of viral RNA. Comfirmation is usually done through presence of specific anti-rubella IgM. When isolation of the virus is necessary, it is obtained from urine and detected as interference with replication of echovirus 11 in monkey cell cultures.
There is no treatment to this day for rubella. The best way to prevent infection is through vaccination with the live cold-adapted vaccine strain of virus. It is usually administered at 2 years of age.

38. Orthomyxoviruses

The family of Orthomyxoviridiae consists of 3 members, influenza viruses A, B and C. Only types A and B are associated with severe human disease. These viruses are enveloped, negative, segmented RNA viruses. Segmented genome facilitates the development of new strains through mutations. This genetic instability is responsible for the annual epidemics and periodic pandemics of influenza infections worldwide.



Influenza virions are pleomorphic, appearing spherical or tubular and ranging in diameter from 80 to 120 nm. The envelope contains two glycoproteins, hemagglutinin and neuraminidase, the membrane protein (m_2) and is internally lined by the matrix protein (M_1). The genome of the influenza A and B viruses consists of 8 different helical nucleocapsid segments, each containing a negative RNA associated with the nucleoprotein and the transcriptase (RNA polymerase components). Influenza C has only 7 genomic segments.

HA binds sialic acid on epithelial cell receptors and promotes cell fusion, NA cleaves sialic acid helping release of the virus from the cells and is the target of antiviral drugs and M_1, m_2 and NP are type-specific viral antigens differentiating influenza A from B and C. The m_2 protein is also a target for antiviral drugs.

Replication begins with binding of HA to cell sialic acid and internalisation of the virus. Once the endosome containing the virus is acidified, a cascade of events takes place, leading to release of the viral genome into the cytoplasm, where it travels to the nucleus (unlike most RNA viruses) and is transcribed into mRNA. Viral proteins then promote binding to the host ribosome and translation begins. Then viral genome segments are enveloped in a random manner, with 8 to 11 segments per virion. The new viruses are released 8 hours after infection.

Influenza initially establishes a local upper respiratory tract infection. It does so by attacking and killing mucous-secreting, ciliated and other epithelial cells, causing loss of the primary defense system. If the infection spreads to the lower respiratory tract, it can cause severe desquamation of bronchial and alveolar epithelium to a single-cell basal layer or to the basement membrane.

Influenza also promotes bacterial adhesion to the epithelial cells. Pneumonia may result form a viral pathogenesis or from a secondary bacterial infection. Low viremia may present but influenza rarely involves organs other than the lungs.

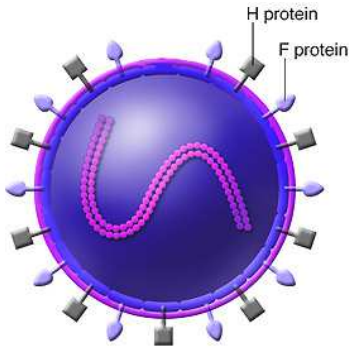
Infection ranges from asymptomatic to severe, depending on the degree of immunity against the infecting strain of virus. Patients with undelying cardiorespiratory disease, immunocompromised patients, pregnant women, elderly patients and smokers are more prone to the severe form.

Diagnosis of influenza is based on the clinical symptoms, the season and the presence of the virus in the community. Laboratory tests are used to distinguish influenza from other

respiratory viruses. They include isolation of the influenza virus on monkey kidney cell cultures. The test involves adding of sheep erythrocytes to the culture and when influenza is present, hemagglutination occurs.
Rapid antigen assays can detect and distinguish influenza A and B. RT-PCR, using generic influenza primers, can be used.
Acetaminophen, antihistamines and similar drugs are used to relieve the symptoms of influenza. The antiviral drug amantadine and its analogue rimantadine inhibit an uncoating step of influenza A virus but don't effect influenza B and C viruses.
Zanamivir and oseltamivir inhibit both influenza A and B enzyme NA.
Airborne spread of influenza is almost impossible to limit. However, protection is brought through immunisation. It can be either natural (resulting from prior exposure) or by vaccination with a killed form of the virus. The downside to vaccination is that is must be done every year, since the viral genome and subsequently the viral strains, mutate and produce new ones.

39. Morbillivirus

The morbilliviruses are a member of the family of Paramyxoviridiae and include the human pathogen measles virus.
Paramyxoviruses consist of negative, single-stranded RNA in a helical nucleocapsid surrounded by a pleomorphic envelope. They are similar to orthomyxoviruses but don't have the segmented genome of the influenza virus.



Measles virus

The nucleocapsid consists of the nucleoprotein (NP), polymerase phosphoprotein (P) and large protein (L). The L protein is the RNA polymerase, the P protein helps in RNA synthesis and the NP protein helps maintain genomic structure. The envelope contains two proteins, fusion (F) and hemagglutinin (H), hemaglutinin-neuraminidase (HN), or G protein.
Replication begins with binding HN, H or G proteins with sialic acid of host cells. The measles virus can bind to CD46 inhibiting the complement system.
Replication occurs in host cell cytoplasm, to which RNA polymerase is carried as part of the nucleocapsid. The genome is transcribed into individual mRNAs and a full-length positive RNA. New genomes associate with L, P and NP proteins to form the nucleocapsid. Mature virions then reach the host cell membrane and exit it.
Measles are one of the five classic childhood exanthems, along with rubella, roseola, fifth disease and chickenpox.

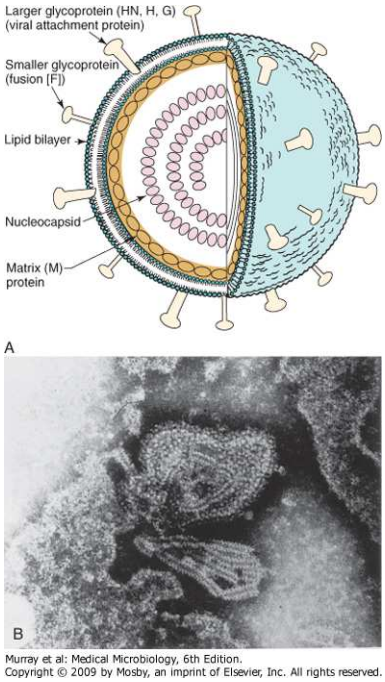
Measles is known for its propensity to cause cell fusion, leading to formation of giant cells. The result is the virus passing freely from cell to cell, avoiding antibody control. Measles is highly contagious and is transmitted from person to person by respiratory droplets. Local replication of the virus in the respiratory tract procedes its spread to the lymphatic system and viremia.
The virus can infect the conjuctiva, respiratory tract, urinary tract, small blood vessels, lymphatic system and CNS. Once a patient is infected and recovers, he or she has lifelong immunity against the virus.
Clinical manifestation of measles is usually so characteristic that laboratory confirmation is rarely needed. Isolation and culture of the measles virus is very difficult. Respiratory and blood specimens should be collected during the prodromal stage and up to 1 to 2 days after appearance of the rash. Measles antigens can be detected in pharyngeal cells or urinary sediment with immunofluorescence. Antibodies, specifically IgM can be detected during the rash phase.
A live attenuated measles vaccine is been given to all children younger than 2 years of age for 50 years, resulting in significant reduction in the incidence of the disease. The vaccine is given in combination with rubella vaccine and mumps vaccine (MMR). No specific antiviral treatment is available for measles.

40. Mumps virus, parainfluenza virus and respiratory syncytial virus

The parainfluenza viruses belong to the family of Paramyxoviruses. They are a group of respiratory viruses, causing mild cold-like symptoms but can also cause serious respiratory tract disease.
They affect epithelial cells of the respiratory tract. The virus replicates faster than the measles or mumps virus and can cause giant cell formation and cell lysis. Viremia is rare with the parainfluenza virus. In only 25% of cases does the virus spread from the upper respiratory tract to the lower.
IgA response is protective but short lived. The virus can manipulate cell-mediated immunity, limiting development of memory.
Parainfluenza viruses 1, 2 and 3 cause a range of symptoms varying from a mild cold-like upper respiratory tract infection to bronchiolitis and pneumonia. Croup may develop in infants (subglottal swelling) closing the airways.
Parainfluenza virus is isolated from nasal washings and respiratory secretions and grows well on primary monkey kidney cells. Like other paramyxoviruses, the parainfluenza virus is labile during transport to the laboratory so caution must be taken.
Treatment of croup involves administration of nebulised cold or hot steam and careful monitoring of the upper airway. No live attenuated vaccine is available and inactivated strains are ineffective in causing antibody production.

The mumps virus, also belonging to the paramyxovirus family, is the cause of acute, benign, viral parotitis (painful swelling of the salivary glands).

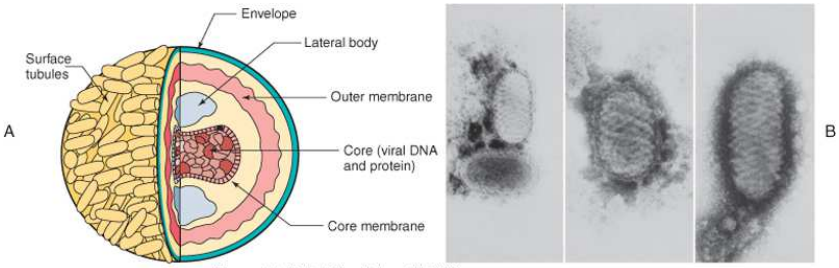
The virus initiates infection in the epithelial cells of the upper respiratory tract and infects the parotid gland. Through viremia, the virus spreads to other places of the body. Infection of the meninges occurs in more than 50% of patients.
Mumps infections are usually asymptomatic. Clinical illness manifests as parotitis and almost always accompanied by fever.



The virus can be recovered from saliva, urine, the pharynx, Stensen's duct and CSF.
A clinical diagnosis can be confirmed by serologic testing. ELISA, immunofluorescence and hemagglutination inhibition can be used to detect the virus, antigen or antibody.
Vaccines provide the only effective means for preventing the spread of mumps infection. Antiviral agents are not available.
Respiratory syncytial virus, or RSV, is a member of the Pneumovirus genus. Unlike other paramyxoviruses, RSV lacks hemagglutinin and neuraminidase. It's the most common cause of fatal acute respiratory tract infection in infants and young children. It infects virtually everyone by the age of 2, with reinfections occuring througout life.
As the name suggests, RSV induces syncytia. The pathologic effect occurs from direct viral invasion of the respiratory epithelium, followed by immunologically mediated cell injury. Necrosis of bronchi leads to formation of plugs of mucus, fibrin and necrotic material within smaller airways.
RSV can cause any respiratory tract illness, from a common cold to pneumonia. Upper respiratory tract infection with prominent rhinorrhea is most common in older children and adults.
The presence of viral genome in infected cells or nasal washings can be detected with Reverse Transcriptase-PCR techniques. Isolation of the virus in cell cultures is difficult.
Ribavirin is administered to patients with an increased predisposition to severe courses of infection (immunocompromised patients). Passive immunisation with anti-RSV Ig is available for premature infants. Infected children must be isolated.

41. Poxviruses, papillomaviruses, polyomaviruses

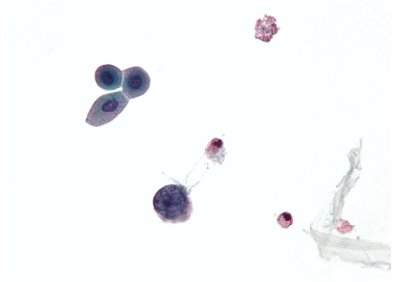
The family of poxviruses includes the human virus variola (small-pox, genus Orthopoxvirus) and molluscum contagiosum (genus Molluscipoxvirus).
Poxviruses are the largest viruses, almost visible on light microscopy. The viral genome consists of a large, double-stranded, linear DNA that is fused at both ends. The replication of poxviruses is unique among the DNA-viruses, as it must encode the enzymes required for mRNA and DNA synthesis, as well as activities other DNA viruses normally obtain from the host cell.



After being inhaled, the small-pox virus replicates in the upper respiratory tract. Dissemination occurs via lymphatic and cell-associated viremic spread. Molluscum contagiosum however is acquired through direct contact with lesions and don't spread extensively, causing a wart-like lesion rather than a lytic infection. A cell to cell spreading of the virus facilitates in avoiding immune control.
Humans are exclusive hosts to the virus and to this day, small-pox have been erradicated. The disease is consistent with visible pustules, covering the whole body. The lesions of molluscum contagiosum differ significantly from pox lesions in being nodular to wart-like. The diagnosis is confirmed histologically by the finding of characteristic large, easinophilic, cytoplasmic inclusions in epithelial cells.
Immunisation with animal poxvirus protects against small-pox.
Both Papillomaviruses and Polyoviruses used to belong to the same family of Papovaviruses but are now considered as individual families.
Human papilloma virus, or HPV, can be distinguished into cutaneous HPV or mucosal HPV, according to the susceptible tissue. The shape of the HPV capsid is eicosahedral and consists of two structural proteins forming 72 capsomeres. The HPV genome is a circular DNA molecule.
The virus is acquired by close contact and infects the epithelial cells of the skin or mucous membrane. The virus persists in the basal layer and then produces viruses in terminally differentiated keratinocytes. The virus causes benign outgrowth of cells into warts. The warts resolve spontaneously, possibly as a result of immune response. Certain types are associated with dysplasia and can become cancerous.
Clinical manifestations of papillomaviruses are skin warts (associated with cutaneous syndromes) and benign head and neck or anogenital tumors (associated with mucosal syndromes). The warts can be plantar, common or flat. Benign tumors consist of laryngeal papillomas, oral papillomas, conjuctival papillomas and cervical intraepithelial neoplasias (cancer).

Hyperplasia of the prickle cells under a microscope can histologically confirm a wart along with an excess production of keratin. DNA molecular probes and PCR from

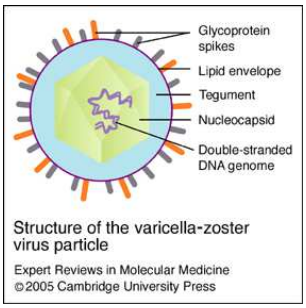
cervical swabs and tissue specimens are the methods of choice for establishing the diagnosis.
Warts spontaneously regress, a process that may take from months to years.
Inflammatory stimulators such as imiquimod, interferon and others can promote healing.
Avoiding to come in direct contact with infected tissue is the best way to prevent spreading from person to person.
The human polyomaviruses (BK and JC viruses) are ubiquitous but usually don’t cause disease and are difficult to grow in cell cultures.
The polyomaviruses are smaller, contain less genome and are less complex than the papillomaviruses. Replication is typical for viruses, with transcriptional and DNA replication machinery provided by a growing cell.



The virus is probably acquired through the respiratory route and spreads by viremia to the kidneys early in life. Infections are asymptomatic. The virus then establishes persistent and latent infection in organs such as the kidney and lungs. In immunocompromised patients, JC virus is activated, spreads to the brain and causes progressive multifocal leukoencephalopathy (PML), where the virus transforms astrocytes and kills oligodendrocytes. The BK virus is ubiquitous but not associated with serious disease.
The diagnosis of PML is confirmed by the presence of PCR-amplified viral DNA in CSF and MRI or CT evidence of lesions. The term leukoencephalopathy refers to the presence of lesions only in the white matter.
There is no specific treatment for polyomavirus infection other than decrease the immunosuppression responsible for virus reactivation and occurance of symptoms.

42. Varicella and herpes zoster virus

VZV causes chickenpox (varicella) and upon recurrence, causes herpes zoster or shingles. As an alpha-herpesvirus, VZV shares many characteristics with HSV, including the ability to establish latent infection of neurons, the importance of cell-mediated immunity in controlling and preventing serious disease and the characteristic blister-like lesions.
VZV has the smallest genome of the human herpesviruses. VZV replicates in a similar manner but slower and in fewer cell types than HSV. Such cells are fibroblasts, activated T cells, epithelial cells and epidermal cells.

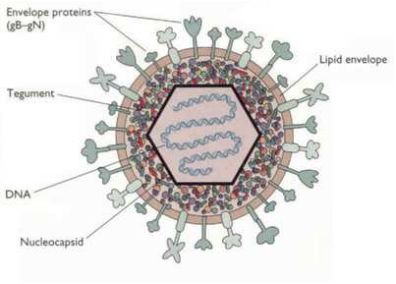


Structure of the varicella-zoster virus particle
Expert Reviews in Molecular Medicine

VZV is generally acquired by inhalation and primary infection begins in the tonsils and mucosa of the respiratory tract. The virus is then transferred in blood and lymph to the cells of the reticuloendothelial systen and in subsequent days, spreads to the whole body and skin. The virus is phagocytosed and is transmitted from cell to cell through cell-cell communication. It then enteres the latent phase in the dorsal root or cranial nerve ganglia after the primary infection. The virus can be reactivated in adults or patients with depressed immunity. On reactivation, the virus is known as herpes zoster.
In children it manifests as chickenpox (varicella), one of the five classic childhood exanthems (along with rubella, roseola, fifth disease and measles). The disease is usually mild in children between the ages of 5 and 9 but in teenagers and adults can be more severe, with potential pneumonia.
A direct fluorescent antibody to membrane antigen test can be used to examine skin lesion scrapings or biopsy specimens. Isolation of VZV isn't routinely done because the virus is labile during transport to the laboratory. Serologic tests that detect antibodies to VZV are used to screen people for immunity to VZV.
Treatment may be appropriate for adults and immunocompromised patients with VZV infections and for people with herpes zoster but children with varicella usually don't need treatment. ACV, famciclovir and valacyclovir have been approved for treatment of VZV infections.
Like other respiratory tract diseases, it's difficult to limit infection. Because the disease is mild in children and provides lifelong immunity, exposure at a young age is often encouraged.
Immunocompromised patients receive protective administration of varicella-zoster immunoglobulin, to avoid severe disease.
A live attenuated strain has been licensed for use and is administered after 2 years of age on the same schedule as measles, mumps and rubella vaccine.

43. Herpesvirus simplex

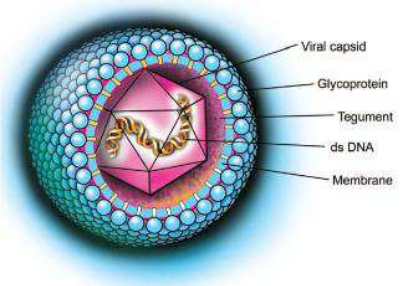
HSV or Herpes Simplex Virus, was the first herpesvirus to be recognised. There are two types: HSV-1 and HSV-2, sharing many common characteristics such as DNA homology, antigenic determinants and disease symptoms.
HSV genome can encode 80 proteins, only half of which are necessary for viral replication. HSV also encodes at least 10 glycoproteins that serve as viral attachment proteins, fusion proteins, structural proteins and immune escape proteins.
HSV can infect almost every cell type of the human body and also those of other organisms. The virus generally causes lytic infections of fibroblasts and epithelial cells and latent infections of neurons. HSV binds to cells through proteoglycans on the outer surface of most cell types. It then penetrates the cell by fusion with its envelope and the virion releases the capsid into the cytoplasm. The capsid attaches to the nuclear envelope and delivers the genome to the nucleus. Viral genome replicates as soon as the polymerase is synthesised. This triggers transcription of the late genes from which structural and other proteins are encoded. The new viruses are released by exocytosis or cell lysis. The virus can also spread between cells through intercellular bridges.



HSV can cause lifelong infection. Recurrent disease is a source of contagion whereas the virus may cause asymptomatic shedding. HSV is transmitted in saliva, vaginal secretions and by contact with lesion fluid. HSV-1 is generally transmitted orally and HSV-2 is transmitted sexually.
Children and sexually active people are mostly at risk for classic HSV-1 and HSV-2 infection, respectively. Physisians, dentists and nurses are at risk of infection when they come in contact with the mouth or genitals and immunocompromised people and neonates are at risk of disseminated, life-threatening disease.
Laboratory diagnosis includes direct microscopic examination of cells from the base of a lesion, cell culture, assay of a tissue biopsy or serological tests, testing for antibodies for epidemiologic studies.
Treatment of HSV-1 and HSV-2 infections includes administration of acyclovir, penciclovir, valacyclovir, famciclovir, adenosie arabinoside or trifluridine. Resistance to these drugs may develop from mutations in viral DNA.
No vaccine is currently available for HSV.

44. Human cytomegalovirus, Epstein-Barr virus, HHV 6,7,8

CMV is a common human pathogen infecting 0,5-2,5% of all newborns and approximately 40% of women visiting clinics for STDs. It's the most common viral cause of congenital defects. Although it causes mild or asymptomatic disease in children and adutls, it's an opportunistic pathogen in immunocompromised patients.
CMV is acquired from blood, tissue and most body secretions. It causes productive infection of epithelial and other cells. The virus can establish latency in T cells, macrophages and other cells. Suppression of cell-mediated immunity allows recurrence and severe presentation of the disease whereas CMV generally causes subclinical infection.
The virus can infect the eyes, lungs, GIT, nervous system, lymphoid system, major organs and neonates. According to the age and health status of the patient, disease can range from asymptomatic to multiple symptomatic.
Laboratory diagnosis is based on histology, cell culture or serological testing (measuring the level of IgM in the blood).
Treatment includes ganciclovir, valganciclovir, cidofovir or foscarnet. Use of condoms during sexual intercourse greatly reduces the spreading of the virus.

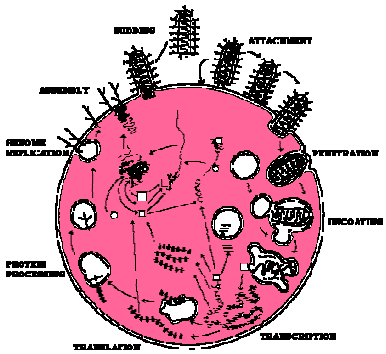


HCMV Human Cytomegalovirus

EBV or Epstein-Barr virus, has developed into the ultimate B lympocyte parasite. EBV causes heterophile antibody-positive infectious mononucleosis and has been associated with endemic Burkitt lymphoma, Hodgkin disease and nasopharyngeal carcinoma.
The virus in saliva initiates infection of oral epithelia and spreads to B cells in lymphatic tissue. There occurs productive infection of epithelial and B cells. The virus promotes growth of B cells, causing T cells to kill and limit this overgrowth. Antibody role is limited. EBV stablishes latency in memory B cells and is reactivated when the B cell is activated. T cells response (lymphocytosis) contributes to symptoms of infectious mononucleosis.
The virus causes lifelong infection, with recurrent disease being the cause of contagion. EBV may also cause asymptomatic shedding. Transmission occurs via saliva, close oral contact ("kissing" disease) or sharing of items such as toothbrushes and cups.
Children experience asymptomatic or mild disease. Teenagers and adults are at risk of infectious mononucleosis whereas immunocompromised patients are at highest risk of life-threatening neoplastic disease.
There is no effective treatment or vaccine available for EBV disease. Control of the infection is difficult but its occurance elicits lifelong immunity. Exposure to the virus early in life is the best mean by which to prevent later serious disease.
The two variants of HHV6, HHV6A ad HHV6B, and HHV7 are members of the genus Roseolovirus of the subfamily beta-herpesviridiae. HHV6 is associated with a common

disease in children, exanthem subitum, commonly known as roseola. HHV7 was later found to also cause roseola.
Infection with HHV6 occurs very early in life. The virus replicates in the salivary glands, is shed and transmitted in saliva. Like CMV, it infects lymphocytes, monocytes, epithelial cells, endothelial cells and neurons. Also like CMV, the virus is likely to be activated in patients with AIDS or other lymphoproliferative and immunosuppressive disorders.
HHV8 or Kaposi Sarcoma-Associated Herpesvirus, is also associated with AIDS and the unique member of the gama-herpesviridae subfamily. Like EBV, the B cells are the primary traget of HHV8 but the virus can also infect a limited number of epithelial cells, monocytes and sensory nerve cells.

45. Rhabdoviridae

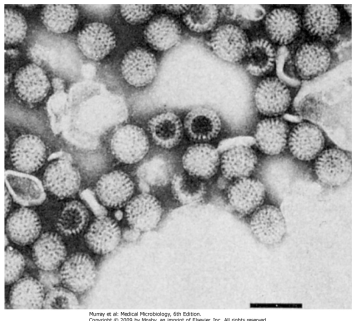


The members of the family Rhabdoviridiae include pathogens of a variety of mammals, fish, birds and plants. The family contains Vesiculovirus, Lyssavirus, an unnamed genus constituting the plant rhabdovirus group and other ungrouped rhabdorivuses.
Rabies virus (Lyssavirus) is the most significant pathogen of this family of viruses. Rhabdoviruses are simple viruses encoding only five proteins and appearing as bullet-shaped, enveloped virions. Inside the envelope, the helical nucleocapside is composed of single-stranded, negative RNA.
For replication, the rabies virus binds either to nicotinic acetylcholine receptors or to the neural cell adhesion molecule of host cells. It’s then endocytosed, the endosome is acidified, uncoating the virus and releasing it into the cytoplasm where replication occurs.
Rabies infection usually results from the bite of a rabid animal. Rabies infection of the animal causes secretion of the virus in its saliva and promotes aggressive behaviour. The virus can also be transmitted by inhalation of aerosoles.
The virus can directly infect nerve endings or muscle at the site of inoculation. It remains at this site for days to months before progressing to the CNS. The virus penetrates the spinal cord through dorsal root ganglia and once this occurs, the brain is rapidly infected. The affected areas are the hippocampus, brain stem, ganglionic cells of the pontine nuclei and Purkinje cells of the cerebellum.

The virus can disseminate from the CNS to the skin, salivary glands, cornea, retina, adrenal medulla, renal parenchyma and pancreatic acinar cells. After brain infection,

encephalitis develops and neuros degenerate. Antibody response isn't stimulated until the late stage of the disease and this can block the progression. Rabis is fatal once clinical disease is apparent. People at highest risk are veterinarians and animal handlers, people bitten by a rabid animal and inhabitants of countries with no pet vaccination programmes. The most characteristic symptoms of rabies is hydrophobia.
Laboratory tests are done to confirm the diagnosis, after a person has been bitten by an animal and shows signs of neurologic disease. The diagnosis is made through detection of viral antigen in the CNS or skin, isolation of the virus, detection of the genome and serologic findings. Rabies antibodies could be detected in the late stage of disease in blood and CSF by the method of ELISA.
Clinical rabies is almost always fatal unless treated. Once the symptoms have appeared, little other than supportive care can be given. Postexposure prophylaxis is the only hope for preventing symptoms from occuring.
The first protective measure is treatment of the wound, which should be immediately washed with soap and water and other substances to inactivate the virus. Subsequent immunisation with vaccine in combination with human rabies immunoglobulin is recommended.
Ultimately, the prevention of human rabies is dependent on effective control in domestic and wild animals, through the removal of stray animals and vaccination of all dogs and cats.

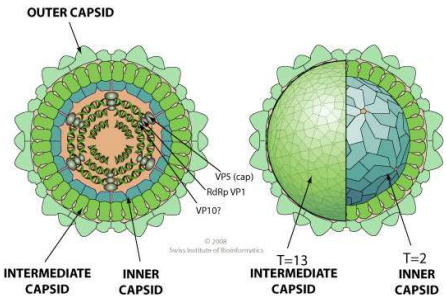
46. Reoviridiae, Coronaviridiae, Rhinovirus



The Reoviridiae consist of orthoreoviruses, rotaviruses, orbiviruses and coltiviruses. They have a double-layered protein capsid containing 10 to 12 segments of double-stranded RNA genome. Orthoreoviruses cause asymptomatic disease in humans. Rotaviruses cause human infantile gastroenteritis, a very common disease.
Depending on the orthoreovirus strain, the virus can be neurotropic or viscerotropic in mice. In humans, if disease manifests, it has mild cold-like symptoms or mild GIT disease symptoms. Laboratory diagnosis is through detection of viral antigen or RNA in clinical material from the throat, nasopharynx and stool. Treatment includes self-resolution of the symptoms.

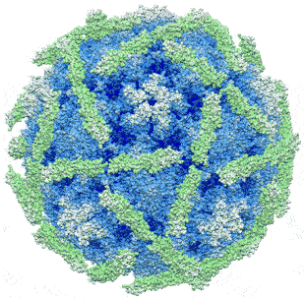
Rotaviruses are common agents of infantile diarrhea worldwide. The virions are relatively stable at room temperature and can withstand a wide range of pH (3,5-10). This means the virus can survive in the stomach after a meal and replication begins

when it’s absorbed by the columnar epithelial cells. Like the cholera toxin, the toxin-like protein of rotaviruses prevents absorption of water and secretion of it in stool.
Direct detection of the virus in stool specimens is sufficient for laboratory diagnosis. Cell culture of the virus is difficult and unreliable for diagnosis.
Rotavirus infections are acquired early in life. There is no specific antiviral therapy but supportive rehydrating therapy is required to restore water and electrolyte loss.



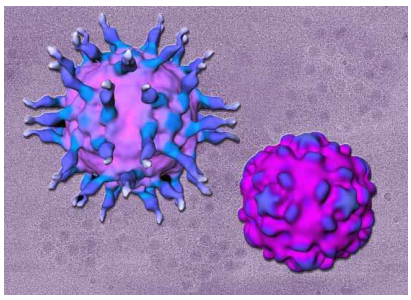
The coltiviruses and orbiviruses infect vertebrates and invertebrates. The coltivirus causes Colorado tick fever and related human disease. It infects erythroid precursor cells. Serious hemorrhagic disease can result from infection of intravascular endothelial and vascular smooth muscle cells. The orbivirus mainly causes disease in animals, including blue-tongue disease in sheep.
Diagnosis of Colorado fever can be established through direct detection of viral antigen on the surface of erythrocytes, viral isolation or serologic tests. No specific treatment is available for Colorado tick fever. The disease is generally self-limited, making supportive care sufficient. Prevention includes avoiding tick-infested areas and use of protective clothing and tick repellents.
Coronaviruses are the second most prelavent cause of the common cold. It’s also linked to gastroenteritis in children and in adults.
The virus is enveloped with the longest positive RNA genome. The glycoproteins on the surface appear club-shaped, giving the virus a halo (corona) appearance around it.
Coronaviruses inoculated into the upper respiratory tract have shown to infect epithelial cells and remains there because the optimum temperature of growth is 33-35 ^{o}C. The virus is most likely spread in aerosols and large respiratory droplets. Infection occur mainly in infants and children.
Laboratory tests are not routinely performed to diagnose coronavirus infections. The method of choice is detection of viral RNA in respiratory and stool samples.
Control of the respiratory transmission of the common cold from coronaviruses is both impossible and unnecessary, since the severity of the disease is very mild.
Rhinoviruses are the most important cause of the common cold. They belong to the family of Picornaviruses. The infection is self-limited and doesn’t cause serious disease. The virus is unable to replicate in the GIT and is labile to acidic pH. Optimal growth temperature is 33 ^{o}C. Transmission is through close contact and inhalation of the virus and people of all ages are at risk. No laboratory diagnosis is necessary since clinical symptoms are very characteristic for the disease.

47. Echoviruses, coxsackieviruses



Echovirus and coxsackievirus belong to the large family of Picornaviruses. They are small, RNA-viruses with a naked capsid structure. Echoviruses got their name from enteric cytopathic human orphan, originally thought not to be associated with disease. Coxsakieviruses got their name from the town of Coxsackie, New York, where they were first isolated. They are subdivided into A and B groups.
The plus-strand RNA of the picornaviruses is surrounded by an icosahedral capsid and the genome resembles an mRNA. The naked picornavirus genome is sufficient to infect a cell. The virus replicates in the host cell cytoplasm and the viral RNA is translated into a polyprotein, which is then cleaved into enzymatic and structural proteins. Most viruses are cytolytic.
Enteroviruses enter via the oropharynx, intestinal mucosa or upper respiratory tract and infect the underlying lymphatic tissue. In the absence of antibodies, enteroviruses spread via viremia to cells of a receptor-bearing target tissue. The infected target tissue determines tha subsequent disease. Coxsackievirus A is associated with diseases involving vesicular lesions (herpangina) whereas Coxsackievirus B is associated with myocarditis and pleurodynia. Echovirus along with both types of Coxsackievirus can also be responsible for aseptic meningitis.
Transmission can be through the fecal-oral route, ingestion of contaminated food or water, contact with infected hands and fomites or inhalation of infectious aerosols. Newborns and neonates are at the highest risk of serious coxsackievirus and enterovirus infection.
CSF can be analysed in the laboratory for enterovirus aseptic meningitis. In contrast with bacterial meningitis, in viral meningitis CSF samples lack neutrophils and the glucose level is usually normal or slightly low.
Echovirus and coxsackievirus are usually isolated from throat or stool specimens and also CSF in patients with meningitis. Coxsackievirus B can grow on primary monkey or human embryo kidney cells. Coxsackievirus A can’t grow on most tissue cultures.
The exact type of enterovirus can be determined by specific antibody and antigen assays (ELISA, immunofluorescence, RT-PCR).
A new antiviral drug called pleconaril can be administered early in the disease and it blocks the penetration of picornaviruses into host cells. There are no vaccines for echovirus or coxsackievirus. Transmission can be reduced by improvements in hygiene and living conditions.

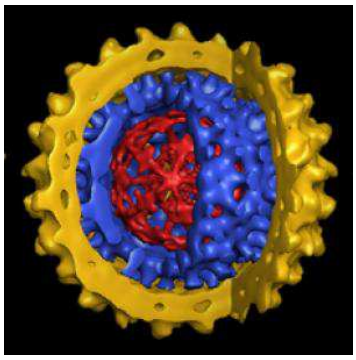
48. Polioviruses



The poliovirus is a member of the family of Picornaviruses. These viruses are small, RNA-viruses with a naked capsid. There are three types of poliovirus: 1, 2 and 3. As given by the name, poliovirus is the causative agent of poliomyelitis.
The plus-strand RNA of the picornaviruses is surrounded by an icosahedral capsid and the genome resembles an mRNA. The naked picornavirus genome is sufficient to infect a cell. The virus replicates in the host cell cytoplasm and the viral RNA is translated into a polyprotein, which is then cleaved into enzymatic and structural proteins. Most viruses are cytolytic.
Wild-type polio infections are rare because of the success of the polio vaccines.
Polioviruses may cause: asymptomatic illness (infection of the oropharynx and gut, 90% of all polio infections), abortive poliomyelitis (minor illness, 5% of infections), nonparalytic poliomyelitis (aseptic meningitis, 1-2% of infections) and paralytic poliomyelitis (major illness, 0,1-2% of infections).
Poliovirus type 1 is responsible for 85% of cases of paralytic polio. In this disease, the virus spreads from the blood to the anterior horn cells of the spinal cord and to the motor cortex of the brain. The severity of paralysis is determined by the extent of neuronal infection and damage.
Young children are at risk of asymptomatic or mild polio whereas older children and adults are at risk of asymptomatic to paralytic poliomyelitis.
Laboratory analysis of CSF from patients with aseptic meningitis can reveal a lymphocytic pleocytosis due to poliovirus. Polioviruses can be isolated from a patient's pharynx during the first days of illness, from the feces but only rarely from CSF. The virus grows well on monkey kidney tissue culture.
The exact type of poliovirus can be determined by specific antibody and antigen assays (ELISA, immunofluorescence, RT-PCR).
There are two types of polio vaccines: inactivated polio vaccine (IPV) and live attenuated oral polio vaccine (OPV). Both incorporate all 3 strains of polio, are stable, are relatively inexpensive and induce a protective antibody response. OPV has the disadvantage of high risk of vaccine-associated poliomyelitis.
Children should receive the IPV at 2 months, 4 months and 15 months and then at 4 to 6 years of age. Alternatively, the first two doses of IPV can be followed by OPV.

49. Hepatitis viruses (A-G)

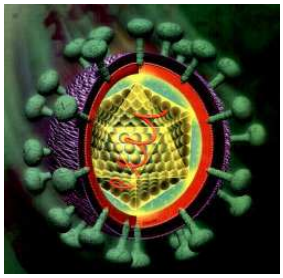
The hepatitis viruses are a group of 6 viruses (A to E and G). Although all primarily infect the liver and the symptoms are similar, the actual viruses differ in structure and replication, transmission, time course and others. Hepatitis viruses A and B are the classic hepatitis viruses, whereas the rest (C, D, E, G) are called non-A non-B hepatitis viruses (NANBH viruses).



Hepatitis A virus causes infectious hepatitis and is spread by the fecal-oral route. Infection often occurs from consumption of contaminated water, shelfish or other food. Hepatitis A virus is a picornavirus, a member of the genus Heparnavirus.
The virus has a naked, icosahedral capsid and a positive single-stranded RNA genome. It is not cytolytic and damage is caused by the immune response. The virus enters through the oropharynx or the intestinal epithelium and targets the parenchymal hepatocytes. It replicates inside the cells and is released in the bile and then stool. The incubation is a few weeks and the onset of disease is abrupt. Usually severity of the disease is mild, with a mortality rate of less than 0,5%. There is no chronicity or carrier state. Laboratory diagnosis is done through detection of anti-hepatitis A virus IgM whereas there is no specific therapy. Protection can be achieved by proper hygiene and vaccination with an inactivated vaccine.
Hepatitis B virus is the major member of the hepadanviruses, of the genus orthohepadanviridiae. It infects the liver and to a lesser extent the pancreas and kidneys of humans and chimpanzees. The viral genome is a small, circular, partly double-stranded DNA. It encodes reverse transcriptase. It causes “serum” hepatitis and affects only hepatocytes. It’s non-cytolytic and damage occurs from the immune response. Transmission is done by parenteral or sexual means. Incubation of the virus is several months and onset of the disease is insidious. The severity of the disease is occasionally severe, with mortality ranging from 1-2%. Chronicity and carrier states are possible. Laboratory diagnosis is done through detection of hepatitis B serum antigen and anti-hepatitis B IgM. Other complications include hepatocellular carcinoma and liver cirrhosis. Active and passive immunisations can prevent infection whereas therapy includes administration of interferon-alpha and lamivudine.

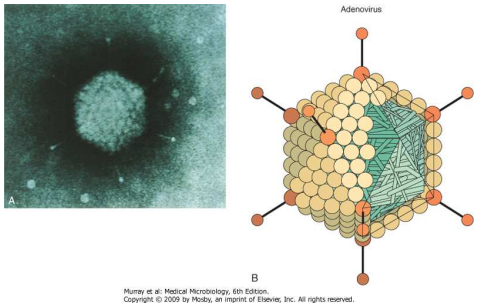
Hepatitis C is the most common cause of NANBH infections and was the major cause of post-transfusion hepatitis before routine blood-screening was performed. It’s a membef

of the Flaviviridiae family, genus Hepacivirus. The virus is enveloped and the genome is a positive, single-strand RNA. It's transmitted like the hepatitis B virus. It also causes no cytolytic effect on hepatocytes and damage occurs from immune response. Antigen variability makes it difficult to be eliminated by the immune system. The disease is milder than hepatitis A and B viruses, with an incubation period ranging from a few weeks to many months. Other complications include hepatocellular carcinoma and liver cirrhosis. Laboratory diagnosis is done by ELISA detection of anti-hepatitis C virus antibodies. Interferon-alpha and ribavirin are administered during therapy whereas a liver transplant may be necessary. There is no vaccine available due to antigenic variability of the virus.



Hepatitis D virus belongs to the genus of Deltoviruses. It is enveloped and has an icosahedral shape and its genome is a circular single-stranded RNA.For its replication is requires presence of hepatitis B virus, making a patient with hepatitis B volrnurable to superinfection with hepatitis D virus. It effects hepatocytes directly, causing a cytolytic effect on them. Disease can be coinfectious, superinfectious or fulminant. Transmission is done by either parenteral or sexual means. Incubation of a few weeks leades to abrupt onset of disease. Chronic or carrier states are possible with mortality being high to very high. Other complications include cirrhosis and fulminant hepatitis. Laboratory diagnosis is done by ELISA detection of anti-hepatitis D antibodies. Immunity against hepatitis B virus prevent hepatitis D infection.
Hepatitis E is usually spread by the fecal-oral route, especially in contaminated water. It is the enteric non-A non-B form of hepatitis. The virus has a capsid and the genome is a naked positive single-stranded RNA. Transmission is similar to hepatitis A virus. In normal patients, the disease is mild but in pregnant women can be severe. No chronic or carrier state is possible. Normal immunoglobulins may not protect against the virus. It's mostly a problem of developing countries.
Hepatitis G virus resembles hepatitis C virus in many ways. It's a flavivirus, transmitted through blood and has a predilection for chronic hepatitis disease. Laboratory diagnosis is done by genome identification by RT-PCR.

50. Adenoviridiae, Parvoviridiae



Adenoviruses are double-stranded DNA viruses. Their genome is linear, double-stranded DNA with a terminal protein. Virions are non-enveloped icosadeltahedrons. The capsule has a fiber, containing the viral attachment protein, that acts as a hemagglutinin. Early proteins promote cell growth and include a DNA polymerase. Replication of the virus takes 32 to 36 hours. It begins by binding of the viral fiber protein to the glycoprotein part of Ig superfamily proteins. This receptor is also called the Coxsackie adenovirus receptor. After being endocytosed, the virus lyses the vesicle and the capsid delivers the DNA to the nucleus, where transcription begins, finally leading to the production of new virions. These virions remain in the cell until it degenerates and lyses, releasing them.

Adenoviruses are capable of causing lytic, latent and transforming infections. These viruses infect epithelial cells of the oropharynx, respiratory tract and GIT. The viral fiber proteins determine the target tissue. Viremia may occur after local replication of the virus and spreading to visceral organs, but usually occurs in immunocompromised patients rather than immunocompetent ones. The virus tends to persist in a latent phase until the host's immune system is compromised or until the patient is infected by another microorganism. Antibodies are important for resolving lytic adenoviruses and prevent reinfection with the specific serotype.

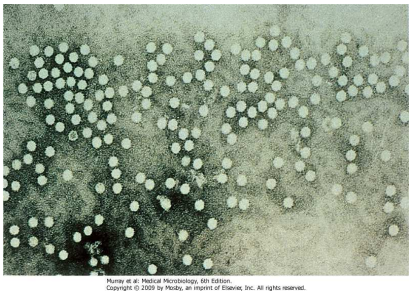
Diseases caused by adenoviruses include acute febrile pharyngitis, pharyngoconjuctival fever, acute respiratory disease, bronchiolitis, conjuctivitis, gastroenteritis with diarrhea, hemorrhagic cystitis (mostly in children) and systemic infections (in immunocompromised patients).

For virus isolation, samples should be taken from the site of primary infection, leading to the disease (e.g. pharyngeal samples are taken from patients with acute pharyngitis, after excluding the possibility of infection by Streptococcus pyogenes). Growth can be accomplished on human embryonic kidney cells and recovery of the virus takes 6 days. Immunoassays, such as fluorescent antibody ELISA, can be performed to detect adenoviruses, without need of isolation. These methods are mostly used for specific serotypes of enteric adenoviruses.

Careful handwashing and chlorination of swimming pools can prevent infection with adenoviruses. There is no approved treatment.

Parvoviruses are the smallest DNA viruses. Only one member of the family is known to cause disease in humans, Parvovirus B19. This specific serotype is responsible for causing erythema infectiosum, or fifth disease, one of the five most common childhood

exanthems.



Parvoviruses are extremely small and have a non-encapsulated, icosahedral capsid. The B19 serotype contains one linear, single-stranded DNA molecule. This virus replicates in mitotically active cells and prefers cells of the erythroid lineage. Once in the cell, the single-stranded DNA is converted into a double-stranded DNA, which is required for transcription and translation.
Studies show that Parvovirus B19 first replicates in cells of the nasopharynx or upper respiratory tract and then spreads through viremia to the bone marrow, where it replicates and kills erythroid precursor cells. This means that the disease has a biphasic course. The initial febrile stage is the infectious stages whereas the second, symptomatic stage is immune mediated.
Even though Parvovirus B19 causes mild disease in children, it can cause polyarthritis in adults, that can last for weeks, months or longer. People with chronic anemia are at high risk of aplastic crisis.
Diagnosis of erythema infectiosum is usually done based on clinical presentation. However, for specific B19 disease to be diagnosed, laboratory tests such as specific IgM or viral DNA detection must be done.
There is no available antiviral treatment or means of control. Vaccines are available for dog and cat parvoviruses.

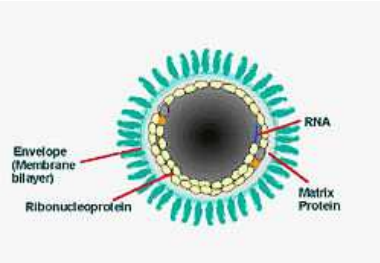
51. Lymphotropic and oncogenic viruses, and other viruses with oncogenic potential

The Oncoviridiae were initially called RNA tumor viruses and have been associated with the development of leukemia, sarcoma and lymphoma in many animals. These viruses are not cytolytic.
Sarcoma and acute leukemia viruses have incorporated modified versions of proto-oncogenes, encoding growth-controlling factors into their genome (v-onc).
The leukemia viruses, including HTLV-1 (Human T-cell Lymphocytic Virus), cause cancer after a long latency period of at least 30 years.
HTLV-1 is cell associated and is spread in cells after blood transfussion, sexual intercourse or breast feeding. The virus infects CD4 T-helper cells and DTH T-cells. These cells are mostly located in the skin, which is why the disease manifests as adult acute T-cell lymphocytic leukemia (ATLL).
HTLV infection is usually asymptomatic but can progress to ATLL in approximately one in 20 persons over a 30 to 50 year period. ATLL of HTLV-1 is a neoplasia of CD4 T-helper cells that can be acute or chronic. ATLL is usually fatal within a year of

diagnosis, regardless of treatment.
HTLV-1 infection is detected using ELISA to find virus-specific antigens in the blood, using RT-PCR for viral RNA or ELISA to detect specific antiviral antibodies.
A combination of azidothymidine and interferon-a has been effective in some patients. However, there is no particular treatment. Prevention of HTLV-1 infection is similar to that of HIV, that is, practice of safe sex and screening of blood supply.
Chronic infections occur when the immune system has difficulty resolving the infection. DNA viruses (except parvoviruses and poxviruses) and retroviruses cause latent infections with the potential of recurrence.
Hepatatis B, C viruses, Epstein-Barr virus, Human Herpes virus 8, papilomavirus and HTLV-1 are associated with human cancers. Epstein-Barr virus usually causes infectious mononucleosis but is also associated with African Burkitt lymphoma, Hodgkin lymphoma and lymphomas in immunosuppressed individuals with nasopharyngeal carcinomas.HTLV-1 is associated with human adult T-cell leukemia.
Direct viral action or the chronic cell damage and repair in livers infected with hepatitis B and C viruses can result in a tumorigenic event leading to hepatocellular carcinoma.
Human Herpes virus 8 produces many cytokines that stimulate cell growth and this growth can progress to Kaposi sarcoma, especially in patients with AIDS.

52. Arboviruses

Arboviruses are a group of viruses from different genera, including Alphavirus (of the family Togaviridiae), Flavivirus (of the family Flaviviridiae) and Bunyavirus (of the family Bunyaviridiae).
Arboviruses are transmitted by arthropods and therefore get their name from this property (arthopod-borne viruses).



Alphaviruses have an icosahedral capsid and a positive, single-strand RNA genome that resembles mRNA. They are slightly larger than picornaviruses and are surrounded by an envelope.
The virus enters the cell by means of receptor-mediated endocytosis. The genome is then delivered to the cytoplasm. There, the genome binds to host cell ribosomes and is translated. Alphaviruses are released on budding from the plasma membrane.

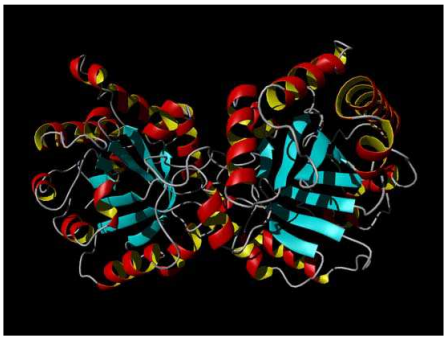
Flaviviruses also have a positive RNA genome and icosahedral capsid, and an envelope but are slightly smaller than an alphavirus. Attachment of these viruses happen in the same way as alphaviruses. The biggest difference between them is that the flavivirus

genome is translated into a single polyprotein.
Infection occurs when an individual is bit from an arthropode such as a mosquito. Female mosquitoes acquire the alphavirus and flavivirus by taking a blood meal from a viremic vertebrate host. A sufficient viremia must be maintained in the vertebrate host to allow acquisition of the virus by the mosquito.
Uppon biting, the mosquito's saliva regurgitates into the victim's bloodstream. The virus then circulates freely in the plasma and infects endothelial cells of the capillaries, monocytes and macrophages.
These viruses are associated with mild systemic disease, encephalitis, arthrogenic disease or hemorrhagic disease.
Both humoral and cellular immunity are elicited and are important to the control of primary infection and the prevention of future infections.
Alphaviruses and Flaviviruses can be grown on both vertebrate and mosquito cell lines, but most are difficult to isolate. Monoclonal antibodies to the individual viruses have become a useful tool for specifying the strain of the virus. Detection of specific IgM antibodies in the serum of a patient is also an indicator of infection.
There is no treatment for these viral infections, other than supportive care.
Bunyaviruses, unlike the other Arboviruses, have a negative, enveloped RNA genome. Apart from mosquitoes, Bunyaviruses can be spread by ticks and flies.
Unlike other negative RNA viruses, Bunyaviruses don't have a matrix protein.
Replication begins with attachment to beta-integrins on the surface of host cells and endocytosis. Like the other Arboviruses, Bunyavirus genome travels to the cytoplasm where it is translated directly by ribosomes of the host cell. Virions are assembled by budding into the Golgi apparatus and are released by cell lysis or exocytosis.
Many Bunyaviridiae cause neuronal and glial damage and cerebral edema, leading to encephalitis. In certain infections, hepatic necrosis may occur.
Detection of viral RNA by RT-PCR has become the accepted method for detecting and identifying bunyaviruses. Specific IgM detection tests are also performed to confirm the diagnosis of bunyavirus infection.
No specific treatment for infections with Bunyaviridiae is available. Prevention includes interruption of contact between humans and the vector, whether arthropod or mammal.

53. Prion infections and slow viral degenerative infections of the CNS

The unconventional slow viruses cause spongiform encephalopathies, which are slow

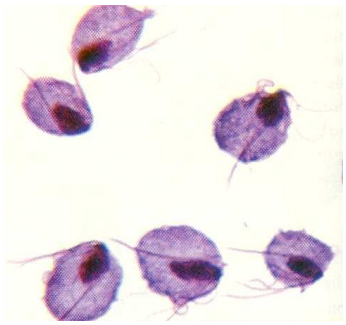
neurodegenerative diseases. These include: Kuru, Creutzfeldt-Jakob disease, variant CJD, Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia and sporadic fatal insomnia.



The slow viral agent is a mutant or conformationally distinct form of a host protein known as a prion (a small proteinaceous infectious particle) which can transmit the disease. The incubation period in humans can last up to 30 years.
In comparison to classic viruses, prions have no nucleic acid, no defined morphology, no proteases, can't withstand temperatures over 80 ^{o}C, cause no cytopathologic effect, have a long incubation period and elicit no immune response, interferon production or inflammatory response.
Prions are imprevious to standard viral disinfection procedures. They can be transmitted via infected tissue or the syndrome can be inhereted. Infection occurs through cuts in the skin, transplantation of contaminated tissues (e.g. cornea), use of contaminated medical devices and by ingestion of infected tissue.
Women and children of a certain tribe in New Guinea were at risk of kuru. Surgeons, transplant and brain-surgery patients and other are at risk of Creutzfeldt-Jakob disease and Gerstmann-Sträussler-Scheinker syndrome.
After clinical signs of the disease manifest, there is a rapid progression to death.
There are no methods for directly detecting prions in tissue though the use of electron microscopy, antigen detection or nucleic acid probes. Also, there are no serological tests detecting antibodies. Initial diagnosis must be done on clinical signs.
No treatment exists for kuru or Creutzfeldt-Jakob disease. Autoclaving at 15 psi for 1 hour or treatment with 5% hypochloride solution can be used for decontamination. Surgical instruments should be carefully disinfected before being reused.

54. Urogenital and intestinal protozoa (T. vaginalis, E. histolytica, G. intestinalis, C. parvum, B. coli)

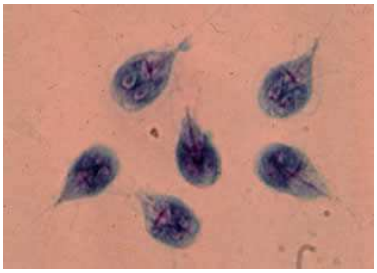
Protozoa may colonise and infect the oropharynx, duodenum, small bowel, colon and urogenital tract of humans. There organisms are transmitted by the fecal-oral route.
Trichomonas vaginalis is not an intestinal protozoan but rather the cause of urogenital infections. The flagellate's four flagella and short membrane are responisble for motility. It exists only as a trophozoite and is found I the urethras and vaginas of women and the urethras and prostates of men.
Most infected women are asymptomatic or have a scant, watery vaginal discharge. Vaginitis may occur with more extensive inflammation and erosion of the epithelial lining. Men are primarily asymptomatic carriers who serve as a reservoir for infections in women.
Microscopic examination of vaginal or urethral discharge for characteristic trophozoites is a diagnostic method of choie. Giemsa or Papanicolaou staining can also be done.
The drug of choice is metronidazole. Because resistance has been reported, retreatment with higher doses may be needed.



Cyst and trophozoite forms of Entamoeba histolytica are detected in fecal specimens from infected patients. Trophozoites can also be found in the crypts of the large intestine. They belong to amoebae of protozoa.
Exposure of the cysts to gastric juice stimulates the release of pathogenic trophozoites in the duodenum. They reproduce in the large intestine and cause local tissue necrosis due to production of a cytotoxin. The types of cells affected are epithelials, neutrophils, lymphocytes and monocytes. Tissue destruction results from the lysis of neutrophils.
Clinical symptoms may vary from asymptomatic carrier state (where cysts enter, are digested and expelled in the stool) to intestinal ameobiasis (mild form of cilitis) to extraintestinal amoebiasis (affecting the liver).
Microscopic examination of stool specimens is insensitive. Extraintestinal amoebiasis can be diagnosed by scanning procedures for the liver. Serologic tests and examination of abscess material can lead to a diagnosis. Detection of fecal antigen by immunologic assays is a new technique for diagnosis.
Acute ameobiasis can be treated with metronidazole, followed by iodoquinol.
Asymptomatic carriage can be eradiacated with iodoquinol or paromomycin.
Chlorination and filtration of water supplies may limit the spread of cysts.

Giardia intestinalis cysts and trophozoites are both detected in fecal specimens from infected patients. They belong to flagellates (mastygophora) of protozoa.
Infection with Giardia intestinalis begins with ingestion of cysts. The minimum human

infective dose is 10 to 25 cysts. Gastric juice stimulates release of pathogenic trophozoites in the duodenum, where they multiply by binary fission. They attach to epithelial cell vili by sucking disks. Inflammation of the epithelium may be observed but frank tissue necrosis doesn't occur. Also, metastatic spread of the parasite to extraintestinal organs is very rare.
Infection with Giardia intestinalis varies from asymptomatic carriers (50% of all infected patients) to symptomatic disease with mild diarrhea to severe malabsorption syndrome.
Stool specimens for cysts and trophozoites should be examined at the onset of diarrhea. However, the presence of many other microorganisms may obscure the the presence of Giardia. This fact means that a negative diagnosis shouldn't be made on a negative stool sample. Detection of fecal antigen by immunologic methods can also be done.
Metronidazole or nitazoxanide are the drugs of choice for eradication of Giardia from both carriers and diseased patients. Accepted alternatives are quinacrine, paromomycin and tinidazole. Boiling of water before use is an effective way to prevent infection in countries with endemic giardiosis.

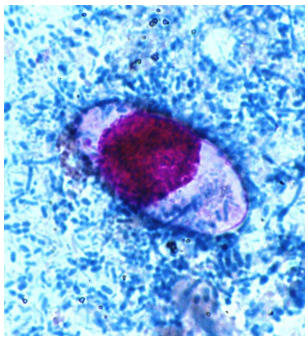


Cryptosporidium parvum belongs to coccidia (sporozoa) of protozoa. They parasitise the brush border of epithelial cells and multiply by gametogony. After production of new infectious oocysts, they may excyst in the GIT and infect new cells or they may be excreted into the environment.
As with other protozoan infection, infection with Cryptosporidium parvum leads either to asymptomatic carriage or a mild, self-limiting enterocolitis characterised by watery diarrhea without blood.
Stool specimens contain oocysts and can be detected microscopically. Specimens can also be stained using modified acid-fast methods or by an indirect immunofluorescent assay.
No broadly effective therapy has been developed for managing Cryptosporidium infections in immunocompromised patients. Spiramycin may control the diarrhea in some patients with early stage AIDS. Nitazoxanide is used to treated children between the ages of 1 and 11 years. Prevention of infection is difficult because of the widespread distribution of the organism. Improved methods for personal hygiene and sanitation should be maintained. Contaminated water should be properly chlorinated and filtered.

Balantidium coli is the only member of the ciliates of intestinal protozoa. The life cycle of this parasite begins with ingestion of infectious cysts, excystation and invasion of trophozoites into the mucosal lining of the large intestine, cecum and terminal ileum.

Trophozoites have many hair-like cilia aiding their movement and a funnel-like primitive mouth called a cytostome.
Asymptomatic carriage of Balantidium coli can exist. Symptomatic disease is characterised by abdominal pain and watery stool with blood and pus. Ulceration of the intestinal mucosa may occur.
Microscopic examination of feces for trophozoites and cysts is performed. Trophozoites are very large and contain a macro and micronucleus. Cysts are smaller. Balantidium coli is a large organism compared to other intestinal protozoa and can be readily detected in fresh, wet microscopic preparations.
The drug of choice is tetracycline. Iodoquinol and metronidazole are alternative antimicrobials. Prevention is the same for amoebiasis.

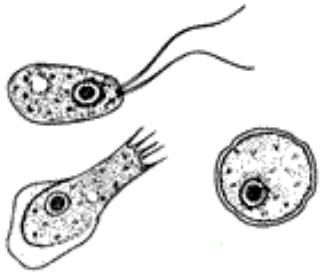
55. Tissue protozoa (T. gondii) and free-living amoebae (N. fowleri, Acantamoeba sp.)



Toxoplasma gondii is a typical coccidian parasite related to Plasmodium, Isospora and other members of the phylum Sporozoa. It’s an intracellular parasite found in a wide variety of mammals and other animals. There is only one species and the essential reservoir host is the common house cat and other felines.
The organisms develop in the intestines of cats, where they are passed into their feces as infectious cysts. These can be ingested by mice and other animals, including humans, causing acute and chronic infections. Tachyzoites, a crescentic type of trophozoites, are responsible for the initial tissue infection and damage.
Most Toxoplasma gondii infections are benign and asymptomatic, with symptoms occuring as the parasite moves to the blood and becomes intracellular. The infection is characterised by cell destruction, production of new organisms and cyst formation. The organism has a particular predilection for cells of the lungs, heart, lymphoid organs and CNS.
Symptoms include chills, fever, headaches and fatigue. In chronic forms hepatitis, encephalomyelitis and myocarditis may develop. Congenital infections occur in infants born to mothers infected with Toxoplasma gondii. In immunocompromised patients reactivation of toxoplasmosis is characteristic.

Antibodies are detected in blood specimens during acute infection. There is a panel of tests called the Toxoplasma gondii serologic profile, determining whether the infection occurred recently or in the past. These include Sabin-Feldman dye to means IgG, ELISA

to measure IgA, IgM and IgE, immunosorbent agglutination assay to measure IgE and differential agglutination to measure IgG. Prenatal diagnosis of toxoplasmosis is done by ultrasonography or amniocentesis. Finding of trophozoites or cysts is a define diagnosis in tissue and body fluids.
Therapy depends on the degree of immunity of the patient and the nature of the infectious process. Immunocompotent patients resolve the disease spontaneously. AIDS patients infected with Toxoplasma gondii are given pyrimethamine plus sulfadiazine. The best alternative is clindamycin in combination with pyrimethamine. Prenatal infection is treated with either clindamycin or spiramycin. Prevention includes routine serologic screening of patients undergone organ transplants. Also, avoiding to come in contact with raw, uncooked meat and cat feces is an effective way in controlling spread.

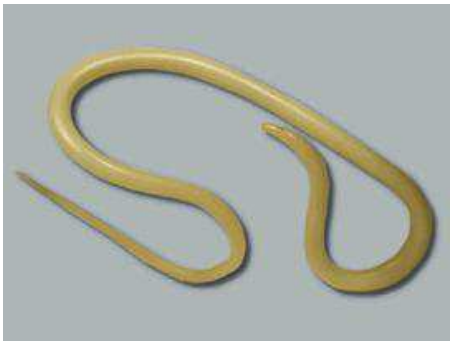


Naegleria and Acantamoeba species belong to free-living amoebae, found in soil and contaminated lakes and other water environments. Humans are infected mostly during the warm summer months while swimming in contaminated water, inhaling cysts from dust or not using sterile cleaning conditions for contact lenses (Acantamoabe infection). Naegleria and Acantamoeba are opportunistic pathogens. Initial colonisation of the nasal passages is asymptomatic and spreading to the brain can occur. Acute primary amoebic meningoencephalitis is the most common disease caused by Naegleria fowleri. In contrast, Acantamoaba produces granulomatous amoebic encephalitis and single or multiple brain abscesses, mostly in immunocompromised patients. Eye and skin infections, such as keratitis, may also occur after infection with Acantamoeba.
For laboratory diagnosis, nasal discharge, CSF and corneal scrapings should be collected. The specimens are observed microscopically using a saline wet preparation and iodine stain. In Naegleria, only amoeboid trophozoites are found whereas in Acantamoeba infections, both cysts and trophozoites are detected.
Treatment of amoebic meningoencephalitis is difficult. The best drug against Naegleria is amphotericin B combined with miconazole and rifampin. Acantamoeba infections may be treated with pentamidine, ketoconazole and flucytosine. Prevention and control of the infection is diffcult because the organisms are widely spread. Banning infected areas from swimming or diving is difficult to enforce.

56. Intestinal nematodes (A. lumbricoides, T. trichiura, E. vermicularis)

Nematodes are the most easily recognised forms of intestinal parasites because of their

large size and cylindrical, unsegmented bodies, hence their common name roundworms. Ascaris lumbricoides are large (20-35cm) pink worms with a complex life cycle. Ingestion of infective eggs releases larval worms that penetrate the duodenal wall, enter the blood stream, carried to the liver and heart and then enter the pulmonary circulation. Here, they multiply within the alveoli and after a period of 3 weeks are coughed up and swallowed to re-enter the GIT. Male and female worms then mature in the jejunum where as many as 200,000 fertilised eggs can be produced per day, for up to a year. Migration of a single adult worm into the bile ducts and liver can cause extensive tissue damage. The worms can also penetrate the intestinal walls and cause peritonitis and secondary bacterial infection.
Stool specimens are tested for eggs. Occasionally, adult worms can pass also into the feces. X-rays of the intestines and cholangiograms of the biliary treet and liver can reveal worms.
The drugs of choice for treatment of symptomatic disease are albendazole or mebendazole.



Trichuris trichuria, commonly known as the whipworm, has a simple life cycle. Ingested eggs hatch into a larval form in the small intestine that travel to the cecum, where they penetrate the mucosa and mature into adults. After about 3 months, the female worm can produce as many as 10,000 eggs per day. Trichuria eggs are distinctive because of their dark bile staining.
The clinical manifestation of trichuriasis is generally related to the intensity of worm burden. A small number of worms causes asymptomatic infection, with the risk of secondary bacterial infection as the heads pass through the intestinal mucosa. Bloody diarrhea with abdominal pain and weakness are characteristic of infection with a large number of larvae. Anemia and eosinophilia are also seen in severe infections.
Stool examination reveals the characteristic bile-stained eggs with polar plugs.
The drug of choice is albendazole or mebendazole. As with Ascaris, prevention depends on education, personal hygiene and sanitation.

Enterobius vermicularis, the pinworm, is a small white worm. Infection begins with ingestion of embryonated eggs. Larvae hatch in the small intestine and migrate to the large intestine, where they mature into adults within 2 to 6 weeks. Females produces

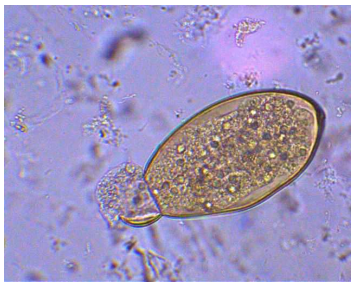
characteristic asymmetric eggs. As man as 20,000 eggs are deposited on the perianal skin.
Many children and adults show no symptoms and serve only as carriers. Patients with allergies against worm secretions experience pruritus and loss of sleep. Worms that migrate to the vagina may cause genitourinary problems and granulomas. Penetration through the bowel wall into the peritoneal cavity, liver and lungs isn't frequent.
Diagnosis of enterobiasis is usually done based on clinical manifestations and confirmed by detection of characteristic eggs on the anal mucosa. An anal swab can also be used to examine for eggs microscopically.
The drug of choice is albendazole or mebendazole. To avoid spreading the disease within the family, treatment of all family members is recommended. Personal hygine and sanitation aid to prevention and control.

57. Nematodes causing larval tissue helminthoses (Toxocara sp., T. spiralis), Trematodes (F. hepatica)

Toxocara species (T. canis and T. cati) are ascarid worms naturally parasitising the intestines of cats and dogs. They may accidentally infect humans, producing disease known as visceral larva migrans (VLM) and ocular larva migrans (OLM). Ingestion of eggs by humans and hatching of larva isn't followed by the normal life cycle. Larvae can penetrate the gut and reach the blood stream where they migrate to various tissues. The larvae can penetrate any tissue of the body, causing bleeding, eosinophilic granuloma formation and necrosis. The organs most frequently affected are the lungs, heart, kidneys, liver, skeletal muscles, eyes and CNS.
Diagnosis of VLM and OLM is done based on clinical findings, eosinophilia, known exposure to cats or dogs and serologic conformation. Examination of feces for eggs isn't useful.
Treatment usually includes symptomatic treatment of the infection, since antiparasitic agents prove unhelpful. Antihelmintic therapy with albendazole or mebendazole is often used. Corticosteroid therapy in patients with severe pulmonary disease may be life saving. This zoonosis may be greatly reduced if pet owners eradicated worms from their animals and clean up pet fecal matter from yards and sandboxes.

Trichinella spiralis, a hookworm, is the most important cause of human trichinosis. The infectious larval form is present in the striated muscles of carnivorous and omnivorous mammals. Infection begins with ingestion of contaminated meat. The larvae then leave

the meat in the duodenum and within 2 days develop into mature adult worms. A female worm can produce 1500 larve in 1 to 3 months. These larvae then move to the blood and are carried to various muscle site throughout the body, where they become encysted. Here, they can stay dormant for years and remain infectious.
As with other parasitic infections, most patients have minimal or no symptoms. Patients with less than 10 larvae per gram of tissue are asymptomatic, those with at least 100 have significant disease (gastroenteritis, muscle pain, eosinophilia) and those with 1000 to 5000 have a very serious course that occasionally ends in death.
Diagnosis is based on clinical findings, known consumption of improperly cooked pork and confirmation comes from laboratory finding of cysts in the implicated meat or muscle biopsy specimens.
Treatment is primarily symptomatic since there are no good antiparasitic agents for tissue larvae. Steroids, along with mebendazole, are recommended for severe symptoms. Proper treatment of pork and other meat help reduce the risk of infection.



Fasciola hepatica, a termatode, is one of the liver flukes. It’s commonly known as the sheep liver fluke. Fasciola hepatica is a parasite of herbivores and humans. Infection in humans begins with ingestion of encysted metacercariae. The larval flukes then migrate through the duodenal wall and cross to the peritoneal cavity, penetrate the liver and enter the bile ducts to become adult worms. About 3 to 4 months after this, the worms begin to produce eggs.
Migration of the larval worm through the liver produces irritation of this tissue, tenderness and hepatomegaly. Pain on the right upper quadrant and eosinophilia are commonly observed. Worm toxic secretions lead to hepatitis, hyperplasia of the epithelium and biliary obstruction.
Stool examination reveals operculated eggs. Examination of the patient’s bile differentiates this trematode from a similar one. Presence of eggs in the bile confirms infection with Fasciola hepatica.
The organism responds poorly to praziquantel. Treatment includes bithionol or triclabendazole. People who live in areas frequented by sheep or cattle should avoid ingestion of watercress and other uncooked aquatic vegetation.

58. Cestodes (T. solium, T. saginata, H. nana, E. granulosus)

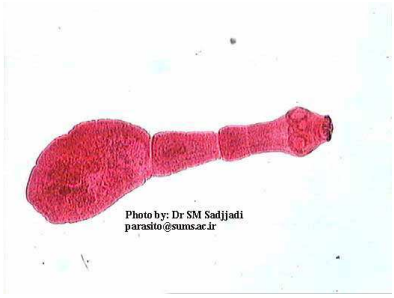
Taenia solium, Taenia saginata, Hymenolepis nana and Echinococcus granulosus all

belong to the family of cestodes, or else tapeworms.
Larval cysts of Taenia solium develop in the intermediate host and have a pear-like apperance. After a human has ingested contaminated pork meat, the larval worms attach to the intestinal wall via four muscular suckers. The worm then produces proglottids. The sexually mature proglottids contain eggs and can contaminate water and food after leaving the host in feces.
Adult Taenia solium in the intestine seldom causes appreciable symptoms. Abdominal discomfort, chronic indigestion and diarrhea may occur. Most patients become aware of the infection only when they see proglottids in their feces.
Stool examination may reveal proglottids and eggs. Because the eggs are identical to those of Taenia saginata, this test isn't sufficient for species determination. Critical examination of the proglottids reveals their internal structure, which is important for differentiation from Taenia saginata.
The drug of choice is niclosamide. Prevention includes thorough cooking of pork of freezing at -20 ^{o}C for at least 12 hours. Sanitation is critical.

The beef tapeworm, Taenia saginata, has a similar life cycle to that of Taenia solium. Cysts are ingested with unproperly cooked beef and after they hatch into larvae, they develop in the small intestine and produce eggs in maturing proglottids. Parasitosis may be for up to 25 years, with the adult worm reaching 10 meters in length. Taenia saginata differs from Taenia solium in that it lacks a crown of hooklets on the scolex.
Patients are generally asymptomatic or may complain of vague abdominal pains, chronic indigestion and hunger pains.
Recovery of proglottids and eggs or the entire worm from feces are enough for a diagnosis. Study of the uterine branches in the proglottids differentiates Taenia saginata from Taenia solium.
Treatment, like in Taenia solium infections, involves niclosamide. Education about proper beef cooking and disposal of human feces is a critical measure.

Hymenolepis nana, the dwarf tapeworm is only 2 to 4 cm in length. Its life cycle is simple and doesn't require any intermediate host.
Infection begins when embryonated eggs are ingested and develop in the intestinal villi

into larval cysts. These larval cysts attach to the intestinal mucosa via four muscular suckers and upon maturation, the adult worm produces egg-laden proglottids. Eggs can pass through the feces and are readily infectious. Hymenolepis nana can also cause autoinfection. Eggs are able to hatch within the intestine, develop into larval cysts and then into full-grown adult worms. This can lead to hyperinfection and severe clinical symptoms.
Only a few worms in the intestine don't cause symptoms. In severe cases, patients experience diarrhea, abdominal pain, headache and other complaints.
Stool examination reveals characteristic Hymenolepis nana eggs with a six-hooked embryo.
The drug of choice is praziquantel. An alternative is niclosamide. Improved sanitation and proper personal hygiene are essential in controlling the transmission.



Echinococcus granulosus infections in humans are accidental. Adult tapeworms are found in nature in the intestines of canines. The larva cyst stage is present in herbivores. The worm consists of a Taenia-like scolex with four suckling disks and three proglottids (mature, immature and gravid). Infective eggs are produced in canine intestines and pass in the feces. If ingested by humans, a six-hooked larval stage hatches, called an oncosphere. It penetrates the intestinal wall and enters the circulation, travelling primarily to the liver and lungs. In humans, the larvae form a unilocular hydatid cyst. Its membrane contains brood capsules where tapeworm heads develop. Cysts accumulate fluid which is toxic and if spilled into the body cavities, can lead to anaphylactic shock and death. The cysts may die and become calcified over long periods.
Because the cysts grow slowly, 5 to 20 years have to pass by before symptoms appear. The expanding cyst may excert pressure on near-by organs, causing damage. In the liver they cause pain and biliary rupture. In the lungs, they lead to coughing, dyspnea and chest pains.
Diagnosis of hydatid disease is difficult. Radiologic examination can reveal presence of cysts. Serologic testing may be useful but results are negative in 10-40% of cases. Clinical symptoms are important in the diagnosis.
Surgical resection of the cysts is the treatment of choice. If the location of the cysts is inoperable, high-dose albendazole or mebendazole is considered. Education regarding the transmission of echinococcosis and the role of canines in the life cycle is crutial for controlling the disease. Personal hygiene and sanitation are also important.

59. Ectoparasites – the causative agents and vectors of infectious diseases

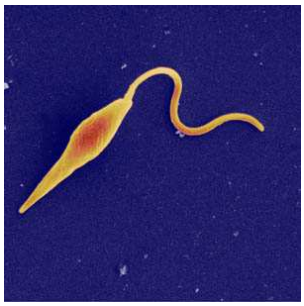
Ectoparasites, or else Arthropods, are the largest of animla phyla with over 1 million

species. The phylum Arthropoda comprises invertebrate animals with segmented bodies, several pairs of jointed appendages, bilateral symmetry and a ridgid exoskeleton.
Most arthropods function indirectly in human disease, they transmit rather than cause it. Of importance is the ability of many arthropods to act as biologic vectors and intermediate hosts in the transmission of bacteria, protozoa, viruses and metazoa.
Out of Chelicerata, mites can transmit scrub typhus and rickettsial pox, carrying the Rickettsia bacteria. Ticks can transmit tularemia (Francisella tularensis), Rocky Mountain fever (Rickettsia rickettsii), Colorado tick fever (Orbivirus), Lyme disease (Borrelia burgdoferi) and Ehrlichiosis (Ehrlichia risticii).
Out of Crustacea, copepods can transmit Diphyllobothriasis (Diphyllobothrium latum) and crabs and various freshwater species can transmit Paragonimiasis (Paragonimus westermani).
Out of Insecta, lice can transmit epidemic typhus (Rickettsia prowazekii), Trench fever (Rickettsia quintana), relapsing fever (Borrelia recurrentis), fleas can transmit the plague (Yersinia pestis), beatles can transmit Hymenolepiasis (Hymenolepis nana), flies can transmit African trypanosomiasis (Trypanosoma brucei) and Leishmaniasis (Leishmania species) and mosquitos can transmit malaria (Plasmodium species), Yellow fever (Flavivirus), Eastern equine encephalitis (Alphavirus), Western equine encephalitis (Alphavirus) and La Crosse encephalitis (Bunyavirus).

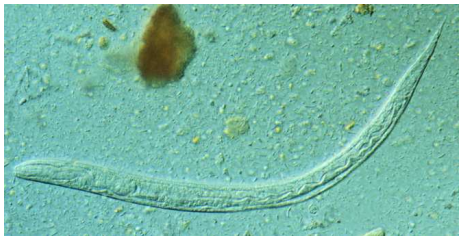
60. Causative agents of selected imported parasitoses (Plasmodium sp., Leishmania sp., Schistosoma sp., D. latum, S. stercoralis)

Plasmodia are coccidian or sporozoan blood parasites. They require mosquitos for sexual reproduction and humans or other animals for asexual reproduction. Plasmodium species is the causative agent of malaria. There are four species that cause malaria: Plasmodium vivax, Plasmodium ovale, Plasmodium malariae and Plasmodium falciparum.
Infection begins when a human is bit by an Anopheles mosquito, which introduces infectious plasmodia sporozoites via its saliva into the parenchymal liver cells, where asexual reproduction occurs (schizogony). This is the exoerythrocytic cycle, lasting 8 to 25 days. The hepatocytes eventually rupture, liberating the plasmodia, which in turn attach to specific receptors on the surface of erythrocytes and enter the cells. Asexual reproduction continues in these cells until they also rupture. This life cycle is then repeated. Plasmodium vivax causes benign fever every 3 days, the same as Plasmodium ovale. Plasmodium malariae causes fever every 4 days. Plasodium falciparum can cause malignant fever every 1 to 3 days, with a possibility of cerebral artery obstruction, leading to death.
Diagnosis is made based on laboratory testing of blood from the patients on the day of the fever. Thin and thick films are collected. Thick films are used to confirm the diagnosis of malaria whereas thin films are used to determine the species responsible. Treatment involves administration of quinine and fluorquinine for extra-cellular parasitosis.
Leishmania are obligate intracellular parasites that are transmitted from animal to human or human to human by bites from a female sand fly.
The promastigote stage is present in the saliva of infected sand flies. They are injected

into the skin with a bite and lose their flagella, enter the amastigote stage and invade reticuloendothelial cells. In here they reproduce and when the host cells rupture, destruction of specific tissues develops (cutaneous, liver and spleen tissues). The life cycle of Leishmania organisms is similar for all species. Leishmaniasis is according to the site of infection and can be cutaneous, mucocutaneous or visceral.
Cutaneous and mucocutaneous forms are diagnosed based on smears from ulcer biopsies and cultures of ulcer tissue. Specimens for diagnosis of visceral leishmaniasis include splenic puncture, lymph node aspirates or liver biopsies. They can be examined microscopically, cultured or subjected to molecular detection methods.
The drug of choice is sodium stibogluconate (Pentostam). Direct injection of fluconazole into the sites of infection on the skin are proven effective. Amphotericin B is the alternative for mucocutaneous infections, instead of stibogluconate. Prevention involves treatment of infections and control of reservoir hosts. Protection from san flies by insect repellents is also essential.



Schistosoma species is a trematode parasite responsible for the tropical infection schistosomiasis or else known as snail fever. The most important species are: Schistosoma mansoni, Schistosoma japonicum and Schistosoma haematobium.
They are obligate intravascular parasites and aren't found in cavities, ducts or other tissues. The infective forms are skin-penetrating cercariae. They enter from the skin to the circulation and develop in the intrahepatic portal circulation or in the visceral, prostatic and uterine venous plexus. As the worm develops, it coats its self with substances that the host recognises as self and there is no immune reaction. After development, male and female worms travel to the mesenteric veins and produce intestinal schistosomiasis or the venous plexus around the urinary bladder and cause vesicular schistosomiasis.
Diagnosis is done by demonstration of characteristic eggs in the feces. They appear golden in colour and the size and shape of their spine is species-identifying.
The drug of choice is praziquantel with the alternative being oxamniquine. Improved sanitation and control of human fecal deposits are critical.
Diphyllobothrium latum is one of the largest tapeworms and belongs to the family of cestodes. It causes diphyllobothriasis is humans after consumption of raw or undercooked fish. Most infections are asymptomatic. Symptoms may include epigastric pain, vomiting and weight loss. On rare occasion it can cause megaloblastic anaemia (a deficiency of vitamin B_{12}).
Examination of stool specimens reveals bile-stained eggs with a knob at the bottom. The drug of choice is niclosamide or paromomycin. Proper cooking of fish greatly reduces the risk of infection.



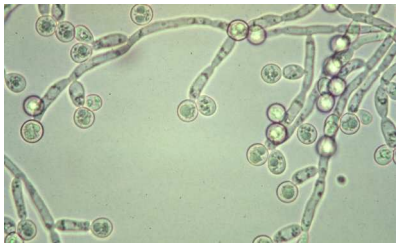
Strongyloides stercoralis, a nematode, can directly enter the skin in a larval form and follows the pulmonary circulation. It's the coughed up and swallowed and adults develop in the small intestine. Females produce a dozen eggs each day which hatch into rhabditiform larvae which are then passed into the stool. In autoinfection, these larvae penetrate the intestinal wall or perianal skin and follow the course of the pulmonary circulation where they are coughed up and swallowed again. This cycle can persist for years and can lead to hyperinfection and even death.
Examination of concentrated stool specimens reveals the larval worms but eggs are generally not seen. Also, culture of the larvae on charcoal or agar plates may be used. Detection of anti-Stronglyloides antibodies in blood is a useful indicator of infection. All infected patients should be treated to avoid autinfection or hyperinfection. The drug of choice is ivermectin. Education, proper sanitation and treatment of infection aid to controlling the disease.

61. Candidoses and other opportunistic mycoses

The most important opportunistic mycoses are caused by: Candida albicans, Cryptococcus neoformans and Aspergillus fumigatus.
Candida species are the fourth most common nosocomial blood stream infections.
All Candida species exist as oval yeastlike forms that produce buds or blastoconidia. Candida albicans also produces pseudohyphae and true hyphae. It also produces germ tubes and terminal, thick-walled chlamydoconidia. In culture, they form smooth, white, creamy, domed colonies. It can also undergo phenotypic switching, where it reversibly changes among diffirent morphotypes.
Disease caused by Candida albicans can be oropharyngeal infection, esophagitis, vulvovaginal infection, skin and nail infections, chronic mucocutaneous candidiasis, urinary tract infection, pneumonia, endocarditis, pericarditis, CNS infection, ocular infection, bone and joint infection, abdominal infection and hematogenous infection.
Laboratory diagnosis is based on collection of the appropriate specimens for direct microscopical examination and culture of the microorganisms. Scrapings of mucosal or cutaneous lesions are treated first with 10% to 20% KOH containing calcofluor white. The yeastlike candidas are seen upon fluorescent microscopy. Culture is done on standard mycologic media or selective chromogenic media such as CHROMagar. Here, Candida albicans appear green and are distinguished from other species of Candida.

Urinary infections are treated either with direct administration of amphotericin B into the bladder or oral administration of fluconazole. Oral fluconazole can also be used to treat more systemic forms of candidiasis, like peritonitis or injected intravenously to

non-neutropenic patients. Prevention is accomplished by treatment of the disease, avoiding of broad-spectrum antimicrobial drugs and proper care of catheters.

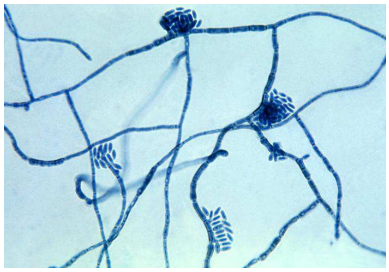


Cryptococcus neoformans, an encapsulated yeastlike fungus, is the causative agent of cryptococciasis, a systemic mycosis. Upon tissue staining with India ink they appear spherical or oval in shape with characteristic halos surrounding the cells.
Diseases caused by Cryptococcus are primary pneumonia on more commonly a CNS infection secondary to a hematogenous and lymphatic spread from primary pneumonia. A 10-15% of patients also experiences skin lesions.
Diagnosis is made based on microscopical examination of blood, CSF or other clinical material. Cultrue on mycologic media will produce mucoid colonies with round, encapsulated, budding yeast cells that are urase-positive. Direct detection of cryptococcal meningitis antigen is also performed in the serum.
All patients should be given amphotericin B plus flucocytosine for 2 weeks, followed by an 8-week consolidation period with fluconazole or itraconazole. AIDS patients need life-long therapy with fluconazole.
Aspergillus species grow in culture as hyaline molds. Grossly, the colonies appear black, brown, green, yellow, white or other colours, depending on the species and growth conditions. Aspergilli grow as branched, septate hyphae that produce conidial heads when exposed to air. In tissue, they stain poorly with HE but are well visualised by PAS, GMS and Gridley fungal stains. The conidial heads are rarely seen in tissue.
Diseases caused by Aspergillus species can be allergic reactions of the upper or lower respiratory tract, colonisation of paranasal sinuses, bronchi or pulmonary cavities, superficial cutaneous infections of wounds or catheters and invasive infections of the bronchi and lungs.
Recovery of the organism from surgically removed tissue accompanied by positive histopathology should be significant for a diagnosis. Most Aspergillus species grow readily on routine mycologic media lacking cycloheximide. Cultural characteristics are used for subsequent species identification. Rapid diagnosis of invasine aspergillosis is based on advanced immunoassays for the Aspergillus galactomannan antigen in serum.
Neutropenic patients are housed in special rooms with air filters to minimise exposure to Aspergillus conidia. Amphotericin B is the drug of choice to treat infections.

62. Superficial, cutaneous and subcutaneous mycoses

Superficial mycoses result from colonisation of the outermost keratinised layers of the

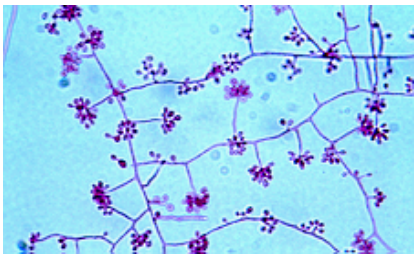
epidermis. Infection elicits little or no immun response.
Pityriasis versicolor is caused by the yeastlike fungus Malassezia furfur and most often involves the upper trunk, extremities, face and neck. Small hypo- or hyperpigmented macules appear on the skin of these areas. Diagnosis is made by direct detection of the fungus under the microscope. Topical azoles or selenium sulfide shampoo are proven effective.
Tinea nigra is caused by the black fungus Hortaea werneckii and manifests as a solitary, irregular, pigmened macule usually on the palms and soles. The infection is not contagious. Diagnosis is easily done by microscopic examination of scrapings treated with 10% to 20% KOH. Azole creams, Whitfield ointment and others involve treatment.
White piedra is a superficial hair infection caused by the yeastlike fungi of the genus Trichosporon. It affects the hairs of the groin and axillae and they appear to have a white to brown swelling along their strand. Trichosporon colonies appear dry, wrinkled and with a cream colour. Microscopy reveals hyphae. Topical azoles are used for treatment.
Black piedra also affects the hair but that of the scalp. The causative agent is Piedraia hortae. Small, dark nodules surround the hair shafts. Examination of the nodule reveals branched, pigmented hyphae. Growth is very slow and presents as a yeastlike colony. A haircut and proper regular washings are enough to treat black piedra.



Cutaneous mycoses involve infections caused by dermatophytic fungi (dermatophytosis) and nondermatophytic fungi (dermatomycosis).
The causative agents of dermatophytoses are the filamentous fungi in the genera Trichophyton, Epidermophyton and Microsporum. They are all able to invade the hair, skin or nails. They are keratinophilic and keratinolytic and are able to break down the keratin surfaces of these structures.
When the hair is infected, the patern of fungal infection can be ectothrix, endothrix or favic. In the ectothrix pattern, arthroconidia are formed on the outside of the hair. In the endothrix they are formed on the inside and in favic, hyphae, arthroconidia and empty spaces resembling bubbles are formed inside the hair.
The classic pattern of dermatophytosis is the “ringworm” pattern of a ring of inflammatory scaling with diminution of inflammation toward the centre of the lesion. Erythema and scaling are also seen. Onychomycoses caused by dermatophytes is seen in about 3% of the world’s population, where the toenails appear thickened, discoloured, raised, friable and deformed.

Specimens of hairs, skin or nails for microbiological examination are treated with 10% to 20% KOH and when observed under the microscope, fungal hyphae are seen.
Cultures are also useful where scraping are placed onto standard mycologic media such as Sabouraud agar and development may take between 7 and 28 days.

Isolated skin infections can be treated with topical azoles (miconazole, clotrimazole). When the hairs and nails are affected, oral antifungals are administered and include griseofluvin, itraconazole and fluconazole.
Onychomycoses caused by nondermatophytic fungi are caused by Scopulariopsis brevicaulis, Scytalidium dimidiatum and other fungi such as Candida, Aspergiullus etc. These types of infections are difficult to treat because the organisms aren't susceptible to most antifungals. Partial surgical removal of the infected nail with oral itraconazole may be useful.

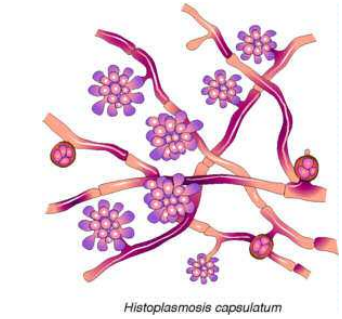


Lymphocutaneous sporotrichosis is caused by Sporothrix schenckii, a dimorphic fungus. Infectio is chronic and characterised by nodular and ulcerative lesions along the lymphatics that drain the primary site of infection. At room temperature, Sporothrix schenckii grows as a mold whereas at 37 ^{o}C is grows as a yeast. Culture takes 2 to 5 days on a variety of mycologic media and confirmation is established by proving the growth from mycelial to yeastlike. Classic treatment includes oral potassium iodide administration in a saturated solution for a period of 3 to 4 weeks.
Chromoblastomycosis is a chronic fungal infection affecting the skin and subcutaneous tissues. Characteristic of this disease are slow-growing verrucous nodules or plaques and is most commonly seen in tropical countries. Causative agents include pigmented fungi of the genera Fonsecaea, Cladosporium, Rhinocladiella and others. Clinical presentations and histopathologic findings are sufficient to diagnose. Antifungal treatment is ineffective. Drugs used include itraconazole and terbinafine. Surgery is not indicated.
Subcutaneous zygomycosis, also known as entomophthoromycosis, is caused by Zygomycetes such as Conidiobolus coronatus (mostly affecting the face of adults) and Basidiobolus ranarum (affecting the proximal limbs in children). Infections occurs by traumatic implantation of the fungus in plant debris in tropical environments. Biopsies of both types are required for diagnosis. Focal clusters of inflammation with eosinophils and zygomycotic hyphae are seen under the microscope. Both infections are treated with itraconazole. Oral potassium iodide is an alternative.

63. Systemic mycoses

Systemic mycoses are caused by dimorphic fungal pathogens, that is, fungi that aquire the form of molds at laboratory temperatures of 20-25 ^{o}C and the form of yeasts at body

temperatures of 37 ^{o}C. These fungi can cause disease in both healthy and immunocompromised patients.
Blastomycosis is a systemic fungal infection caused by Blastomyces dermatitidis. The yeast cells are spherical and multinucleated and produce blastoconidia. They are found in decaying organic material and cause pulmonary diseases (50% of patients), skin, bone, urinary tract and CNS diseases and disseminated disease in patients with suppressed immunity. Microscopic examination of specimens reveals the fungi. Amphotericin B is used in life-threatening or meningeal disease. Itraconazole is used for mild or moderate disease, with fluconazole being and alternative.
Coccidiomycosies is caused by the fungi Coccidioides immitis and Coccidioides posadasii. The yeast cells appear spherical and contain endospores. They are found in soil and dust and cause mostly asymptomatic pulmonary infection (60% of patients) and progressive pulmonary infection and dissemination (skin, bones, joints, meninges) in immunocompromised patients. Culture of sputum specimens reveals mold with barrel-shaped arthroconidia. Antibodies can be detected along with antigens in the urine. Asymptomatic patients don't require treatment but immunocompromised patients need administration of amphotericin B.


Histoplasmosis capsulatum

Histoplasmosis capsulati is caused by Histoplasma capsulatum. The yeast cells appear small, oval and narrow-based. They are found in soil with high nitrogen content (bird or bat droppings) and cause asymptomatic pulmonary infection mostly (90% of patients) and disseminated disease in immunocompromised hosts and children. Sputum or blood cultures reveal mold with tuberculate macroconidia. Antibodies can be detected along with antigens in serum and urine. Asymptomatic patients don't require treatment but severe pulmonary disease requires immediate amphotericin B administration followed by a 12-week period of oral itraconazole.
Penicilliosis marneffei is caused by the fungus Penicillium marneffei. The cells appear elongated, sausage-like intracellular yeasts. They are found in soil and bamboo rats and cause disseminated infection in AIDS patients resembling histoplasmosis or tuberculosis. Blood cultures appear as molds with red pigments. Amphotericin B with or without flucytosine is the treatment of choice. AIDS patients require lifelong treatment with itraconazole.