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DEPARTMENT OF MICROBIOLOGY

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MEDICAL MICROBIOLOGY

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MEDICAL MICROBIOLOGY

UNIT I Introduction to Medical Microbiology

Significance of Microbiology in Medicine, Classification of medically important microbes, Normal microbial flora of the human body: normal flora of skin, eye, throat, gastrointestinal tract and urogenital tract - Infections- Sources, types – opportunistic, nosocomial and community acquired infections - Mode of transmission, carriers and their types – investigation of epidemic diseases.

Unit II Medical Bacteriology

Morphological, cultural and biochemical characteristics of and epidemiology, mechanism of bacterial pathogenesis, lab diagnosis, prophylaxis and control of medically important diseases caused by*: Staphylococcus aureus,* Group A Streptococci, *Corynebacterium diphtheriae, Clostridium tetani, Bacillus anthracis, Leptospira interrogans, Treponema pallidum, Mycobacterium tuberculosis, Escherichia coli, Vibrio cholerae, Niesserriae, Haemophilus influenza, Helicobacter pylori, Pseudomonas* and *Salmonella.* Brief note on Chlamydia, Rickettsia Mycoplasama, anaerobic bacterial infections, Atypical Mycobacterium, Zoonotic bacterial pathogens, Antibiotic susceptibility test: Kirby – Bauer disk diffusion method.

Unit III Medical Mycology

Morphological and cultural characteristics of and epidemiology, mechanism of fungal pathogenesis, lab diagnosis and treatment of medically important diseases caused by*:* Superficial mycosis – *Tinea versicolor*. Cutaneous mycoses: *Microsporum*, *Trichophyton*, *Epidermophyton*. Subcutaneous mycoses: Sporotrichosis, Chromoblastomycosis, Zygomycosis. Systemic Mycoses – *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Cryptococcus neoformans*, *Coccidioides immitis*, *Paracoccidioides brasiliensis*. Opportunistic mycoses: Candidiasis, Cryptococcosis and Aspergillosis. Antifungal susceptibility testing.

Unit IV Medical Virology

General properties of and epidemiology, pathogenesis, lab diagnosis and treatment of medically important viral diseases caused by: Influenza viruses, Measles, Mumps, Rubella, Chicken Pox, Hepatitis A,B,C, Dand E, Poliomyelitis, HIV, Human Papilloma Virus, Rabies, Yellow fever, Dengue and Japanese Encephalitis viruses. Brief note on oncogenic viruses.

Unit V Medical Parasitology and emergence of antibiotic resistant pathogens

Morphology of, and pathogenesis, laboratory diagnosis and treatment of medically important protozoan diseases caused by: *Entomoeba histolytica, Giardia lamblia, Trichmonas vaginalis, Plasmodium vivax*, *Leishmania donovani*, *Taenia solium, Ascaris lumbricoides, Ancyclostoma duodenale* and *Wuchereria bancrofti.* Brief note on the emergence of MDR bacterial, fungal pathogens, extremely drug resistant (XDR) pathogens and superbugs.

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MEDICAL MICROBIOLOGY
UNIT-1

Medical microbiology is a branch of medical science concerned with the prevention, diagnosis and treatment of infectious diseases. In addition, this field of science studies various clinical applications of microbes for the improvement of health. There are four kinds of microorganisms that cause infectious disease: bacteria, fungi, parasites and viruses, and one type of infectious protein called prion.

History

Microbial diseases have undoubtedly played a major role in historical events, such as the decline of the Roman Empire and the conquest of the New World.

In 1347, plague or Black Death struck Europe with a brutal force.

The Roman philosopher Lucretius (about 98–55 BC) and the physician Girolamo Fracastoro (1478–1553 AD) suggested that disease was caused by invisible living creatures.

In 1676, Anton van Leeuwenhoek observed bacteria and other microorganisms, using a single-lens microscope of his own design.

In 1796, Edward Jenner developed a method using cowpox to successfully immunize a child against smallpox. The same principles are used for developing vaccines today.

Following on from this, in 1857 Louis Pasteur also designed vaccines against several diseases such as anthrax, fowl cholera and rabies as well as pasteurization for food preservation.

In 1867 Joseph Lister is considered to be the father of antiseptic surgery. By sterilizing the instruments with diluted carbolic acid and using it to clean wounds, post-operative infections were reduced, making surgery safer for patients.

Germ theory of disease

Pasteur proved that all life even microbes arose only from their like and not *de novo*.. Pasteur had not only resolved the controversy by 1861 but also had shown how to keep solutions sterile.

Robert Koch: The Founder of Koch's Postulates

The first direct demonstration of the role of bacteria in causing disease came from the study of anthrax by the German physician Robert Koch (1843–1910).

Koch used the criteria proposed by his former teacher, Jacob Henle (1809–1885), to establish the relationship between *B. anthracis* and anthrax, and he published his findings in 1876 briefly describing the scientific method he followed.

In this experiment, Koch injected healthy mice with a material from diseased animals, and the mice became ill.

After transferring anthrax by inoculation through a series of 20 mice, he incubated a piece of spleen containing the anthrax bacillus in beef serum in 1876.

The bacilli grew, reproduced, and produced spores.

When the isolated bacilli or spores were injected into mice, anthrax developed.

During Koch's studies on bacterial diseases, it became necessary to isolate suspected bacterial pathogens.

His criteria for proving the causal relationship between a microorganism and a specific disease are known as Koch's postulates in 1884.

‣ Koch's postulates

Koch's postulates (criteria) were useful to prove the claim that a microorganism isolated from a disease was indeed causally related to it. A microorganism was accepted as the causative agent of infectious disease, only when it satisfied all the following criteria:

1. The microorganism must be present in every case of the disease but absent from healthy host.

2. The suspected microorganism must be isolated and grown in a pure culture from lesions of the disease.

3. The isolated organism, in pure culture, when inoculated in suitable laboratory animals should produce a similar disease.

4. The same microorganism must be isolated again in pure culture from the lesions produced in experimental animals.

The specific antibodies to the bacterium should be demonstrable in the serum of patient suffering from the disease. This was an additional criterion that was introduced subsequently.

Most of the human bacterial pathogens satisfy Koch's postulates except for those of *Mycobacterium leprae* and *Treponema pallidum*, the causative agent of leprosy and syphilis, respectively.

Both these bacteria are yet to be grown in cell-free culture media.

Solid medium for culture of bacteria

Koch pioneered the use of agar as a base for culture media. He developed the pour plate method and was the first to use solid culture media for culture of bacteria in 1881.

This development made possible the isolation of pure cultures that contained only one type of bacterium and directly stimulated progress in all areas of bacteriology.

Koch also developed media suitable for growing bacteria isolated from the body. Because of their similarity to body fluids, meat extracts and protein digests were used

as nutrient sources. The result was the development of nutrient broth and nutrient agar media that are still in wide use today.

By 1882, Koch had used these techniques to isolate the bacillus that caused tuberculosis in humans. Koch also discovered that cholera was caused by *Vibrio cholera* in 1883.

He invented the hot air oven and steam sterilizer, and also introduced methods to find out the efficacy of antiseptics. There followed a golden age of about 30–40 years in which most of the major bacterial pathogens were isolated.

- **Koch's phenomenon**

Koch's phenomenon is a hypersensitivity reaction against tuberculosis bacilli demonstrated in guinea pigs. This was first demonstrated by Koch, who showed that guinea pigs already infected with tubercle bacillus, on challenge with tubercle bacillus or its protein developed an exaggerated inflammatory response. **He is Father of Medical Microbiology.**

A major milestone in medical microbiology is the Gram stain. In 1884 Hans Christian Gram developed the method of staining bacteria to make them more visible and differentiable under a microscope. This technique is widely used today.

In 1929 Alexander Fleming developed the most commonly used antibiotic substance both at the time and now: penicillin.

DNA sequencing, a method developed by Walter Gilbert and Frederick Sanger in 1977, caused a rapid change the development of vaccines, medical treatments and diagnostic methods. Some of these include synthetic insulin which was produced in 1979 using recombinant DNA and the first genetically engineered vaccine was created in 1986 for hepatitis B.

In 1995 a team at The Institute for Genomic Research sequenced the first bacterial genome; *Haemophilus influenzae*. A few months later, the first eukaryotic genome was completed. This would prove invaluable for diagnostic techniques.

Discovery of important bacterial agents causing human diseases

S.No	SCIENTIST	YEAR	BACTERIA
1	Hansen	1874	*Mycobacterium leprae*
2	Koch	1876	*Bacillus anthracis*
3	Neisser	1879	*Neisseria gonorrhoeae*
4	Ogston	1880	*Staphylococcus aureus*
5	Loeffler	1884	*Corynebacterium diphtheria*
6	Fraenkel	1886	*Streptococcus pneumonia*
7	Weichselbaum	1887	*Neisseria meningitides*
8	Bruce	1887	*Brucella melitensis*
9	Kitasato	1889	*Clostridium tetani*
10	Yersin	1890	*Yersinia pestis*

Role of Microbiology in the Pharmaceutical and Medical Device

Overview

Microbiology is the study of microorganisms such as bacteria, protozoa, fungi and similar organisms that can't be seen with the naked eye. The need to study these minute organisms started when scientists discovered the association of microbes to specific diseases. The roles of microbiology on the advances in the healthcare industry, especially in pharmaceutical and medical industry have led to great discoveries, from vaccines to devices.

1. Pharmaceutical Industry

Understanding the principles of microbiology and human cell mechanisms allows pharmacists to discover antimicrobial drugs that would prevent an escalating number of communicable diseases. Pharmacists and microbiologists work synergistically to ensure that drug therapies target the opportunistic microbes without harming its human host. Another important role in pharmaceuticals is the use of microbes for the medically important studies, such as Bacteriorhodopsin (figure 2 - adapted from Lehninger Principles of Biochemistry 5th edition), a protein from the plasma membrane of Halobacterium salinarum.

2. Medical Devices

Microbiology plays a significant role in medical devices, such as fluorescent fusion, which are used for fast and precise detection of pathogens in tissue samples. It is a technology for carrying out immunofluorescence studies that may be applied to find specific cells in complex biological systems.

3. Antibiotics

Antibiotics are natural substances that can be used to fight bacterial infections. They are produced and secreted naturally by bacteria and fungi. Different biotechnological techniques are used to produce antibiotics in pure forms and large quantities so they can be used for treating bacterial infections. Antibiotics do not affect viruses.

The search for antibiotics began in the late 19th Century when it was accepted that some diseases are caused by tiny organisms.

The first antibiotic-producing organism was discovered by Alexander Fleming in 1929 - apparently by accident. The antibiotic-producing mould 'contaminated' and killed some bacterial cultures that were part of another scientific experiment. Instead of throwing the contaminated dish away, Fleming examined the mould. He identified the mould as *Penicillium notatum* and named the substance that it produced, penicillin.Fleming found that it was effective against many types of bacteria. Penicillin was extracted and purified only in the mid-20th century. Today it is still the main infection-fighting antibiotic. The development of penicillin-resistant bacteria now limits its effectiveness.

Penicillin kills bacteria by preventing the formation of their cellular walls. Pre-existing cells are unaffected, but all newly-produced cells grow abnormally and are very fragile and easily destroyed in a process called osmotic lysis.

The success of penicillin led to the search for other antibiotic-producing microorganisms, especially from soil environments. Today, several hundreds of antibiotics have been isolated from different microorganisms but only a few of them are clinically useful. The reason for this is that only compounds with **selective toxicity** can be used clinically. Selective toxicity means that an antibiotic will destroy only some organisms. Clinically useful antibiotics must be effective against pathogens but have minimal toxicity to humans and human beneficial microflora. In practice, this is expressed in terms of the **therapeutic** index - the ratio of the toxic dose to the therapeutic dose. The larger the index, the better its therapeutic value is.

Many of the antibiotic-resistant genes of some pathogenic bacteria are carried on their **plasmids - circular DNA strands containing non-essential genetic**

information. This genetic information can be exchanged in a process called conjugation.

4. Vaccine

The name 'vaccine' comes from the Latin 'vacca' which means 'cow' because the very first vaccine was created for smallpox using cows infected with this disease.

Pathogenic microorganisms like bacteria and viruses, can make people sick by destroying body cells or by releasing harmful toxins that destroy body cells. When these same pathogens are 'served' to humans in the form of a vaccine, they can help the body to develop immunity against them. A vaccine is a mixture of dead or weakened pathogens used to induce the formation of antibodies against this pathogen.

Vaccines work by triggering the body's immune response without making it sick. In cases where disease is caused by toxins produced by pathogens, the vaccine contains its weakened toxin. A vaccine is introduced to the body either by injection or within a small drink.

According to the type of pathogens used in the mixture, all vaccines are divided into four main groups:

- Live vaccines are made from live but 'disabled' pathogens. This type of vaccine produces long-term immunity.
- Inactivated vaccines are made of dead pathogenic organisms. Inactivated vaccines produce short-term immunity.
- Toxoids are made of toxic substances produced by pathogenic organisms.
- A subunits mixture is not made of whole pathogenic organisms, but their fragments.

The process of vaccine distribution is called vaccination.

Significance

By nature, our cells fight microbes that enter our body and this is commonly exhibited by pus formation and inflammation of wounds. Macrophages play an important role in immune system because they are capable of ingesting microbes that enter our body through open wounds. However, microbes could adapt and mutate rapidly, which results to opportunistic infectious diseases, such as HIV. On the contrary, microbes can also help us in ways like the way the "good bacteria" lactobacillus functions in our digestive system.

Normal Flora

- Microbes that colonize the human body during birth or shortly thereafter, remaining throughout life, are referred to as normal flora.
- Normal flora consists of different bacteria/fungi that are permanent residents of a certain human body site. They have low virulence, meaning they are non pathogenic in their usual body site, but if they move from this site they can become pathogenic and cause infection (especially in immunocomprised people).
- Normal flora can be found in many sites of the human body including the skin (especially the moist areas, such as the groin and between the toes), respiratory tract (particularly the nose), urinary tract, and the digestive tract (primarily the mouth and the colon). On the other hand, areas of the body such as the brain, the circulatory system and the lungs are intended to remain sterile (microbe free).
- A positive host-microbe relationship is usually described as either mutualistic or commensalistic.
- In mutualism both the host and the microbe benefit. Which is in contract to commensalisms, where one partner of the relationship benefits (usually the microbe) and the other partner (usually the host) is neither benefited nor harmed.

- Mant sites of human body are free from microbes. These include the cerebrospinal fluid, blood, urinary bladder, uterus, fallopian tubes, middle ear, para nasal sinuses and Kidneys.

Types of Normal flora

Divided into two categories namely,

1. **Resident flora. 2. Transient flora**

1. Resident flora:

It consists of relatively fi xed types of microorganisms regularly found in a given area at a given age; if disturbed, it promptly reestablishes itself.

2. Transient flora:

It consists of nonpathogenic or potentially pathogenic microorganisms that inhabit the skin or mucous membranes for hours, days, or weeks.

If the resident microbiota is disturbed, transient microorganisms may colonize, proliferate and produce disease.

There are four reasons to acquire knowledge of the normal flora,

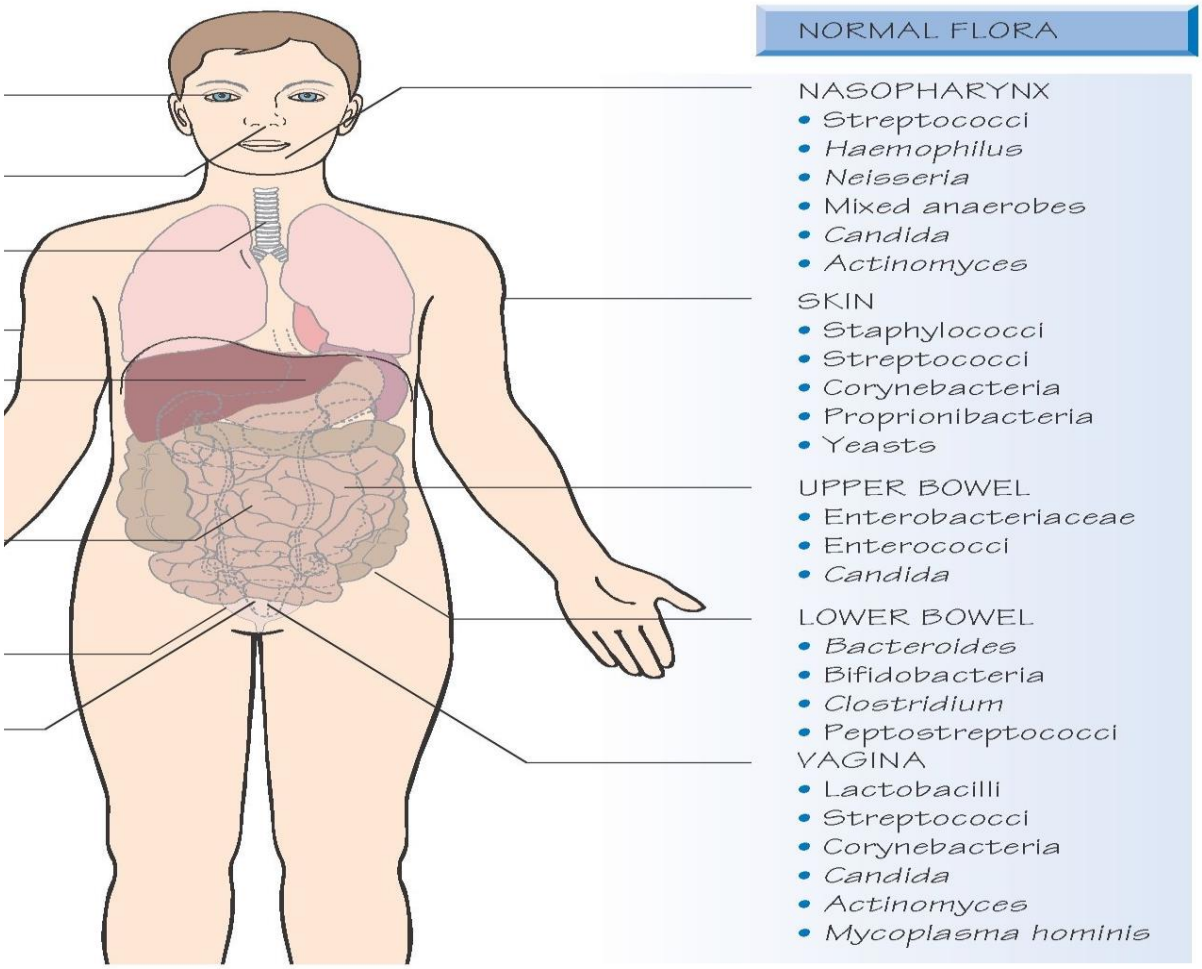
1. To study interactions between resident and transient flora.
2. Help physicians to evaluate the nature of infections.
3. To know what kind of microbes present in and on our body.
4. Increased awareness about normal flora.

Some examples:

- **Skin** – *S. epidermidis*
- **Nose** – *S. aureus*
- **Mouth** – Viridans Streptococci
- **Dental plaque** – *S. mutans*
- **Throat** – Viridans *Streptococci*
- **Colon** – *Bacteroides fragilis, E.coli*
- **Vagina** – *Lactobacillus, E.coli,* Group B *Streptococci*

Life on the Surface, the Skin

- Human skin is not a particularly rich place for microbes to live. The skin surface is relatively dry, slightly acidic and the primary source of nutrition is dead cells.
- *Propionibacterium acnes* are a Gram positive bacterium that inhabits the skin. *P. acnes* causes the common skin condition called acne but not life threatening.
- Another prominent member of the skin flora is *Staphylococcus epidermidis*. This is a highly adapted Gram positive bacterium that can survive at many sites throughout the body. *S. epidermidis* was actually not considered a serious threat to human health prior to the introduction of catheters and surgery.



A Bacterial Sneeze, the Nose

- The human nose is home to the infamous Gram positive bacterium *Staphylococcus aureus*, infections of this bacterium are now a very serious threat to human health because it has become resistant to all commercially available antibiotics.

Conjunctiva

The predominant organisms of the conjunctiva are diphtheroids, *S epidermidis,* and nonhemolytic streptococci. Neisseriae and gram-negative bacilli resembling haemophili (*Moraxella* species) are also frequently present. The conjunctival flora is normally held in check by the flow of tears, which contain antibacterial lysozyme.

A Mouthwash Away

- It's estimated that 500-600 different kinds of bacteria thrive on mucus and food remnants in the mouth. A predominant member of this community is the Gram positive bacterium *Streptococcus mutans*. It grows on biofilms on the surface of teeth (plaque) where it consumes sugar and converts it to lactic acid.
- *Streptococcus pneumoniae* is a much more threatening bacteria that can colonize the mouth.

Braving Stomach Acid

- Gram negative bacterium called *Helicobacter pylori* living in the human stomach.
- *H. pylori* produce an enzyme called urease to convert urea produced by the stomach into ammonia and carbon dioxide.
- The concentration of bacteria in the small intestine remains relatively low (10^6 bacteria per ml) and human enzymes carry out most of the digestion processes.

- This slower flow rate gives bacteria in the colon time to reproduce, it reach very high concentrations like 10^{12}-10^{13} bacteria per ml.

Small Intestine vs. the Colon

- *E. coli* within the colon produce vitamin K, which the human body requires for the process of blood clotting.

Vaginal

- The predominant bacterial species are *Lactobacillus* in the vaginal tract. It appears to have a protective role of women.

Functions

1. Bacteria perform physiological, nutritional and protective functions in the human body.
2. Maintaining a balance is crucial.
3. Explore greater than 99% the microbial world in body site, that to embark on a "second human genome project" where the genomic sequences of the microbes making up our normal flora are determined.

Host microbes interaction

The host-pathogen interaction is defined as how microbes or viruses sustain themselves within host organisms on a molecular, cellular, organismal or population level.

On the molecular and cellular level, microbes can infect the host and divide rapidly, causing disease and homeostatic imbalance in the body, or by secreting toxins which cause symptoms to appear.

Host: An organism harbouring another organism or organisms on or in itself.
Microbes: A microbe is a living thing that is too small to be seen with the naked eye. The human body is home to microbes from all the microbial categories like Bacteria, Fungi, Viruses and Parasites.
Interaction: The combined effect of two or more independent variables acting simultaneously on a dependent variable.

Viruses can also infect the host with virulent DNA, which can affect normal cell processes and evading the immune response.

Depending on how the pathogen interacts with the host, it can be involved in one of three host-pathogen interactions.

1. Commensalism is when the pathogen benefits while the host gains nothing from the interaction.
   Examples: *Bacteriodes sps*, which resides in the human intestinal tract but provides no known benefits.
2. Mutualism occurs when both the pathogen and the host benefit from the interaction, as seen in the human stomach.
   Examples: Many of the bacteria aid in the breaking down of nutrients for the host, and in return, our bodies act as their ecosystem.
3. Parasitism occurs when the pathogen benefits from the relationship while the host is harmed.
   Examples: Unicellular *Plasmodium falciparum* parasite which causes malaria in humans.

Stages of bacterial pathogenesis

1. Transmission from external source into portal of entry.

2. Evasion of primary host defense like skin or stomach acid.
3. Adherence to mucous membrane (usually by pili).
4. Colonization by growth of bacteria at site of adherence.
5. Disease symptoms caused by toxins product or invasion (accompanied by inflammation).
6. Host response (non-specific + specific), during steps 3, 4 and 5.
7. Progression and resolution of disease.

A pathogen must be able to perform the following functions,

1. Invasiveness
2. Infectivity
3. Pathogenesis

1. Invasiveness

Invasiveness is the ability of a pathogen to invade tissues. Invasiveness encompasses,

(I) transmission of microbes

(II) mechanisms for colonization (adherence and initial multiplication),

(III) production of extracellular substances ("invasins"), that promote the immediate invasion of tissues and

(IV) ability to bypass or overcome host defense mechanisms which facilitate the actual invasive process.

I. Transmission of infections

Mode of transmission

1) Human-to-human

a) **Direct contact** – intimate contact, sexual passage through birth.

b) **No direct contact** – faecal-oral (ingestion of contaminated food etc), finger to mouth.

c) **Transplacental** – across placenta, from mother to baby.

d) **Blood-borne** – blood transfusion, intravenous drug use.

e) **Vertical transmission:** from mother to baby through birth canal, breast milk).

f) **Horizontal transmission:** all other pathways.

2) Non human-to-human

a) **Soil source** – spores in soil enter wounds.

b) **Water source** – bacteria in water aerosol are inhaled into lungs.

c) Animal source

- **Directly** – bacteria enter through cat scratch.
- **Via insect vector** – bacteria enter via tick bite.
- **Via animal feces** – bacteria in cattle feces ingested in undercooked hamburgers.

d) **Fomite source** – bacteria in object e.g. towel --- transferred to skin.

II. Adherence & Colonization

Bacterial Adherence to Mucosal Surfaces.

In its simplest form, bacterial adherence or attachment to a eucaryotic cell or tissue surface requires the participation of two factors: a **receptor** and a **ligand**.

The receptors so far defined are usually specific carbohydrate or peptide residues on the eucaryotic cell surface.

The bacterial ligand, called an **adhesin**, is typically a macromolecular component of the bacterial cell surface which interacts with the host cell receptor. Adhesins and receptors usually interact in a complementary and specific fashion with specificity comparable to enzyme-substrate relationships and antigen-antibody reactions.

Table is a list of terms that are used in microbial adherence to surfaces or tissues.

S.No	Adherence factor	Description
1	Adhesin	A surface structure or macromolecule that binds a bacterium to a specific surface
2	Receptor	A complementary macromolecular binding site on a (eucaryotic) surface that binds specific adhesins or ligands
3	Lectin	Any protein that binds to a carbohydrate
4	Ligand	A surface molecule that exhibits specific binding to a receptor molecule on another surface
5	Mucous	The mucopolysaccharide layer of glucosaminoglycans covering animal cell mucosal surfaces
6	Fimbriae	Filamentous proteins on the surface of bacterial cells that may behave as adhesins for specific adherence
7	Common pili	Same as fimbriae
8	Sex pilus	A specialized pilus that binds mating procaryotes together for the purpose of DNA transfer
9	Type 1 fimbriae	Fimbriae in *Enterobacteriaceae* which bind specifically to mannose terminated glycoproteins on eucaryotic cell surfaces
10	Type 4 pili	Pili in certain Gram-positive and Gram-negative bacteria. In *Pseudomonas*, thought to play a role in adherence and biofilm formation
11	Biofilm	exopolysaccharide or slime produced by bacteria that attaches imbedded cells to a surface
12	S-layer	Proteins that form the outermost cell envelope component of a broad spectrum of bacteria, enabling them to adhere to host cell membranes and environmental surfaces in order to colonize.
13	Glycocalyx	A layer of exopolysaccharide fibers on the surface of bacterial cells which may be involved in adherence to a surface. Sometimes a general term for a bacterial capsules.
14	Capsule	A detectable layer of polysaccharide (rarely polypeptide) on the surface of a bacterial cell which may mediate specific or nonspecific attachment
15	Lipopolysaccharide (LPS)	A distinct cell wall component of the outer membrane of Gram-negative bacteria with the potential structural diversity to mediate specific adherence. Probably functions as an adhesin
16	Teichoic acids and lipoteichoic acids (LTA)	Cell wall components of Gram-positive bacteria that may be involved in nonspecific or specific adherence

Portals of entry

The next stage of microbial infection is **colonization**: the establishment of the pathogen at the appropriate portal of entry. Pathogens usually colonize host tissues that are in contact with the external environment.

Sites of entry in human hosts include the

a) urogenital tract, e.g. gonorrhea, syphilis, urethritis.
b) the digestive tract, e.g. typhoid fever, cholera, dysentery.
c) the respiratory tract, e.g. pneumonia, meningitis, tuberculosis.
d) the Skin, e.g. tetanus, Rocky Mountain spotted fever.

- can be active penetration of host's mucous membranes or epithelium membranes or epithelium
- can be passive penetration – e.g., skin lesions, insect bites, wounds
- once below mucous membrane, bacterium can spread to deeper tissues
  - ❖ involves production of specific products and/or enzymes that promote spreading

Invasion substances

Spreading Factors" is a descriptive term for a family of bacterial enzymes that affect the physical properties of tissue matrices and intercellular spaces, thereby promoting the spread of the pathogen.

- **Collagenase** is produced by *Clostridium*. It breaks down collagen, the framework of muscles, which facilitates gas gangrene due to these organisms.
- **Neuraminidase** is produced by intestinal pathogens such as *Vibrio cholerae* and *Shigella dysenteriae*. It degrades neuraminic acid (also called sialic acid), an intercellular cement of the epithelial cells of the intestinal mucosa.
- **Hemolysins,** notably produced by *Staphylococci* (i.e., alpha toxin), *Streptococci* (i.e.,streptolysin) and various clostridia, may be channelforming proteins or phospholipases that destroy red blood cells and other cells by lysis.

2. Infectivity

Infectivity has been shown to positively correlate with virulence. This means that as a pathogen's ability to infect a greater number of hosts increases, so does the level of harm it brings to the host.

Growth and multiplication depends on its degree of infectivity.

Growth and Multiplication of the Bacterial Pathogen

- occurs when pathogen finds appropriate environment within host
- Temperature, pH, oxygen, nutrients
  - Soluble nutrients are in limited supply (sugars,amino acids, organic acids)
  - Vitamins and growth factors are not always unavailable
  - Trace elements (e.g. Fe) may also be in short supply
  - Host produces transferrin proteins that bind Fe, keeping it away from pathogens.
  - Some pathogens produce siderophores which can remove the Fe from the tranferrin
- some bacteria invade specific cells
- some actively growth in blood plasma
  - bacteremia – presence of viable bacteria in blood
  - septicemia – presence of bacteria or their toxins in bloodstream

❖ often environmental factors control expression of virulence genes,

- e.g., *Corynebacterium diphtheriae*
  - gene for diphtheria toxin regulated by iron
- e.g., *Bordetella pertussis*
  - expression of virulence genes increased at body temperature

- e.g., *Vibrio cholerae*
  - gene for cholera toxin regulated by pH, temperature and other factors

3. Pathogenesis

- Pathogenicity
  - The ability of a microbe to cause disease
- Virulence
  - The degree of pathogenicity in a microorganism

Virulence

- Virulence is determined by invasiveness, toxicity, and other factors and other factors produced by a pathogen.
- Various pathogens produce proteins that damage the host cytoplasmic membrane, causing cell lysis and death.

Pathogenesis of Bacterial Diseases

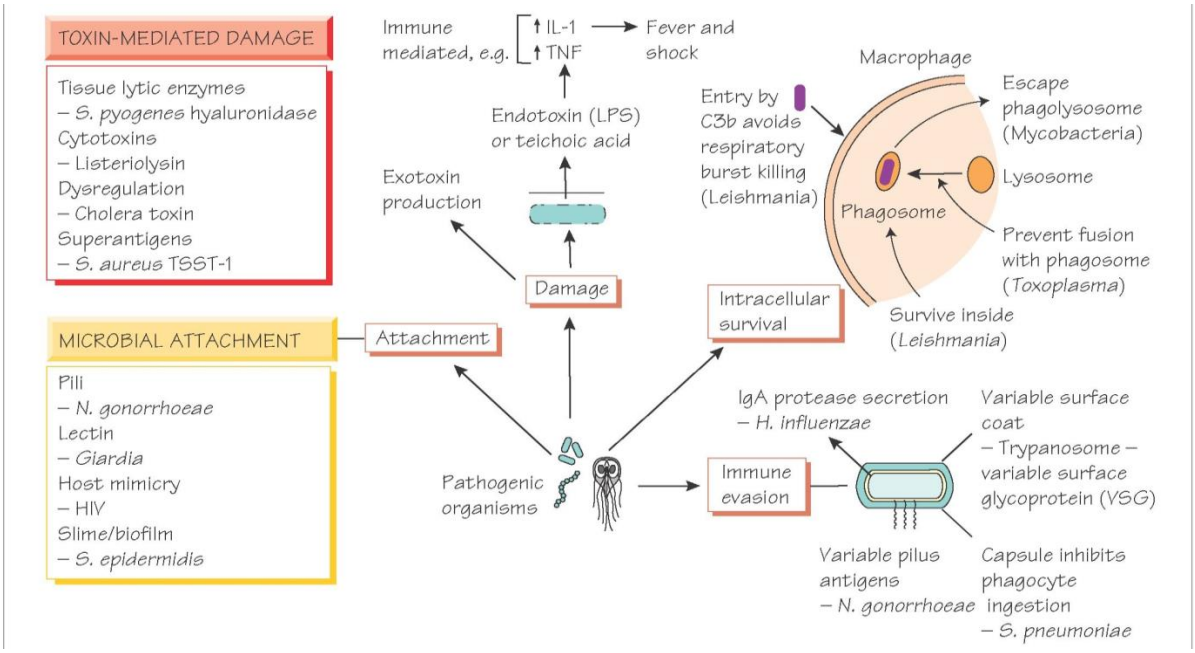
- initially evade host defenses
  - Some pathogenic bacteria are inherently able to resist the bactericidal components of host tissues. components of host tissues.
  - Most successful pathogens, however, possess additional structural or biochemical features which allow them to resist the main lines of host defense
- damage host
  - The ability to invade tissues
  - The ability to produce toxins
- leave host and return to reservoir or enter new host

Toxigenicity

- intoxications
  - diseases that result from entry of a specific preformed toxin into host
- toxin
  - specific substance that damages host
- toxemia
  - condition caused by toxins in the blood of host

Toxin production.

Is the second major way bacteria cause disease.



Exotoxins

- Produced by both *G+ and G- bacteria*
- They are *polypeptides* secreted by certain bacteria and alter specific cells functions resulting in symptoms of disease.
- Found in plasmids or bacterial Lysogenic viruses.
- Most toxic substance known.
- They are antigenic and induce antibodies called **antitoxins**.
- They have an A (active) subunit is an enzyme (ADP ribosylation) + has toxic activity and B (binding) subunit.
- They have different mechanism of action and different targets within the cell and therefore cause many different diseases with characteristic symptoms; some act by *proteolytic cleavage* of cell component, while others act as *superantigens* causing overproduction of cytokines.
- When the exotoxins are treated with formaldehyde (or acid/heat), they convert into **toxoids** which are used in protective vaccines because they keep their antigenicity but have lost their toxicity.

1. Neurotoxins

- are exotoxins that act on nerves or motor endplates to cause paralysis e.g. tetanus toxin, botulinum toxin.

2. Enterotoxins

- are exotoxins that act on GI tract to cause diarrhea. They inhibit NaCl absorption and activate NaCl secretion or mediate destruction of epithelial cells. End result is fluid accumulation in intestines --- diarrhea.

Enterotoxins cause 2 disease manifestations:

a) **Infectious diarrhea ---** bacteria colonize and bind to the GI tract and continuously release their enterotoxins until they are killed by the immune system or by antibiotics.

b) **Food poisoning ---** bacteria grow in food and release enterotoxins in food, the eneterotoxin is then ingested leading to diarrhea and vomiting.

3. Pyrogenic exotoxins

- stimulate release of cytokines and cause rash, fever and toxic shock syndrome.

4. Tissue invasive exotoxins

- allow bacteria to destroy and funnel themselves through tissues. These induce enzymes that destroy DNA, collagen, fibrin, NAD, RBC and WBC.

5. Miscellaneous exotoxins

- are the main virulence factors of many bacteria – can cause disease unique to the individual bacteria.

Endotoxins

- Only in G- rods and cocci.
- They are part of the cell wall (so not secreted!).
- are **lipopolysaccharides (LPS).**
- They all produce the same general effect: *fever and shock*.
- Weakly antigenic they produce weak antibodies, so multiple episodes may occur.
- They don't produce toxoids, so not used in vaccines.
- They are the cause of septic shock, seen in intensive care units

In septic shock it s the bacteria itself that is circulating in the blood, while in toxic shock it is the toxin --- so blood cultures are positive for septic shock, but negative for toxic shock.

Bacterial Virulence factors.

Virulence: *is a quantitative measure of pathogenicity*. It is measure by the number of organisms needed to cause disease.

50% lethal dose (CD50) – is the number of organisms needed to kill half of the hosts.

50% infectious dose (ID50) – is the number of organisms needed to cause infection in half of the host.

The infectious dose of a bacterium depends on their virulence factors. For example if their pilus allows them to adhere well to the mucous membranes, whether they produce exotoxins or endotoxins, whether they have a capsule, if they can survive the different non-specific host defense like acids in stomach

Adherence to cell structures

1. Pili

- Is the main mechanism of adherence to human cells.
- They extend from the surface of bacteria and mediate attachment to specific receptors on cells.

2. Glycocalyx

- Is a polysaccharide "slime layer" secreted by some strains of bacteria.
- It mediates strong adherence to certain structures like heart valves, prosthetic implants and catheters.

3. Fimbria

- Binding to tissue

The different molecules that mediate adherence to cell surfaces are called **adhesins**.

Invasion and intracellular survival.

Invasion of tissue is enhanced by enzymes secreted by bacteria e.g. **hyaluronidase** and **collagenase** secreted by *S. pyogenes* degrades *hyaluronic acid* in subcutaneous tissue --- this allows the organism to spread rapidly.

1. IgA protease

- Degrades secretory IgA – allowing bacteria to attach to mucous membrane e.g. in mouth.

2. Coagulase

- accelerates the formation of a fibrin clot from its precursor form --- it clots plasma.

3. Capsule

- surrounds the bacteria
- antiphagocytic.

4. M-protein

- found in cell wall of G+ cocci --- antiphagocytic.

5. Protein A

- founds in cell wall of G+ cocci
- binds to IgG and *inhibits complement binding to bacteria*.

Intracellular survival - once the bacterium is inside the body it protects itself by avoiding attack by macrophages and neutrophils;

1. Inhibits fusion of phagosomes with lysosomes – like this is avoids the enzymes of the lysosome.
2. Inhibits acidification of phagosomes – reduces the activity of lysosmal degredative enzymes.
3. Escapes from phagosomes in cytoplasm where there are degredative enzymes.

Leucocidin (by *S. aureus*) – kills macrophages and leukocytes.

Toxin production.

Is the second major way bacteria cause disease.

Exotoxins

- Produced by both *G+ and G- bacteria*
- They are *polypeptides* secreted by certain bacteria and alter specific cells functions resulting in symptoms of disease.
- Found in plasmids or bacterial Lysogenic viruses.
- Most toxic substance known.
- They are antigenic and induce antibodies called **antitoxins**.
- They have an A (active) subunit is an enzyme (ADP ribosylation) + has toxic activity and B (binding) subunit.
- They have different mechanism of action and different targets within the cell and therefore cause many different diseases with characteristic symptoms; some act by *proteolytic cleavage* of cell component, while others act as *superantigens* causing overproduction of cytokines.
- When the exotoxins are treated with formaldehyde (or acid/heat), they convert into **toxoids** which are used in protective vaccines because they keep their antigenicity but have lost their toxicity.

1. Neurotoxins

- are exotoxins that act on nerves or motor endplates to cause paralysis e.g. tetanus toxin, botulinum toxin.

2. Enterotoxins

- are exotoxins that act on GI tract to cause diarrhea. They inhibit NaCl absorption and activate NaCl secretion or mediate destruction of epithelial cells. End result is fluid accumulation in intestines --- diarrhea.

Enterotoxins cause 2 disease manifestations:

a) **Infectious diarrhea ---** bacteria colonize and bind to the GI tract and continuously release their enterotoxins until they are killed by the immune system or by antibiotics.

b) **Food poisoning ---** bacteria grow in food and release enterotoxins in food, the eneterotoxin is then ingested leading to diarrhea and vomiting.

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- In septic shock it s the bacteria itself that is circulating in the blood, while in toxic shock it is the toxin --- so blood cultures are positive for septic shock, but negative for toxic shock.

- Septic shock causes death of patient even though the antibiotics have killed the bacteria in the blood. This is because septic shock is mediated by cytokines like **TNF** and **IL-1** that continue to act even thought the bacteria that induced them has been killed.

- The toxic part of LPS is **Lipid A ---** causes the overproduction of cytokines.
- Biological effects of endotoxins include:

1) **Fever** – due to release of endogenous pyrogen (IL-1) by macrophages --- act on temp. center in hypothalamus.

2) **Hypotension** – leads to shock and decreased perfusion of essential organs, caused by bradykinin which increases vascular permeability.

3) **Disseminated Intravascular Coagulation (DIC)** – due to activation of coagulation system through Hageman factor leading to thrombosis and tissue ischemia leading to organ failure.

4) **Activation of alternative pathway of complement cascade** leading to inflammation and tissue damage.

5) **Activation of macrophages ---** increasing their phagocytic ability and activating antibody production.

Difference between Exotoxins & Endotoxins

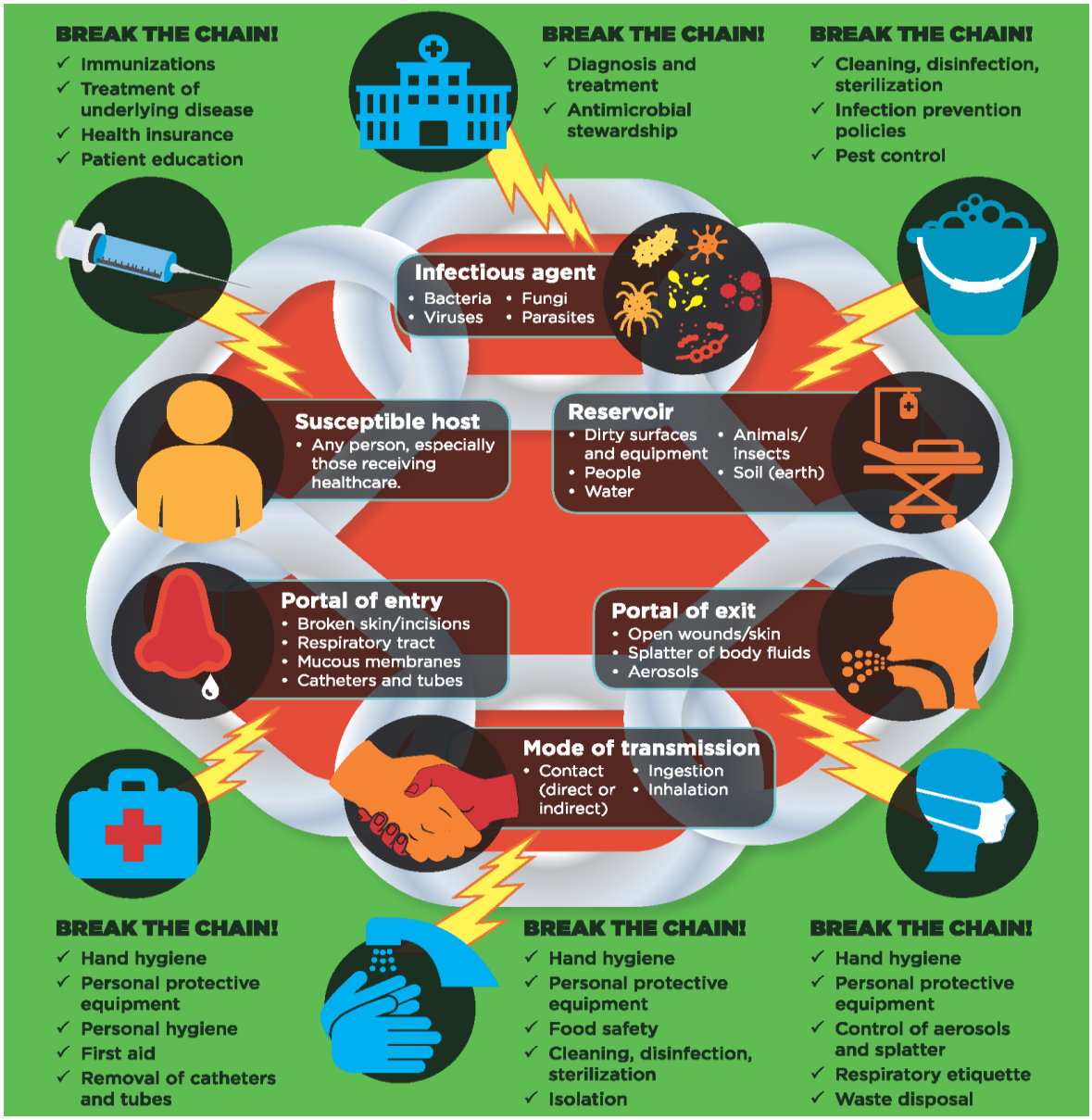
Properties	Exotoxins	Endotoxins
Source	Some bacteria of G+ and G- bacteria	Cell wall of G- bacteria
Secreted from cell	Yes	No
Chemistry	Polypeptide	Lipopolysaccharide
Location of genes	Plasmid or bacteriophage	Bacterial chromosome
Toxicity	High (fatal dose at 1μg)	Low (fatal dose at hundreds of μg)
Clinical effect	Various	Fever and shock
Mode of action	Various	Induces TNF and IL-1
Antigenicity	Induce high antibody titer, antibodies called antitoxins	Poorly antigenic
Vaccines	Toxoids used as vaccine	No toxoids formed and no vaccine available
Heat stability	Destroyed rapidly at 60% (except staphylococcal enterotoxin)	Stable at 100C for 1 hour
Typical disease	Tetanus, botulism, diphteria	Meningococcemia, sepsis by G- rods

Infectious disease cycle

There are many different germs and infections inside and outside of the healthcare setting. Despite the variety of viruses and bacteria, germs spread from person to person through a common series of events.

The six links include: the infectious agent, reservoir, portal of exit, mode of transmission, portal of entry, and susceptible host.

1. **Infectious agent** is the pathogen (germ) that causes diseases
2. **Reservoir** includes places in the environment where the pathogen lives (this includes people, animals and insects, medical equipment, and soil and water)
3. **Portal of exit** is the way the infectious agent leaves the reservoir (through open wounds, aerosols, and splatter of body fluids including coughing, sneezing, and saliva)
4. **Mode of transmission** is the way the infectious agent can be passed on (through direct or indirect contact, ingestion, or inhalation)
5. **Portal of entry** is the way the infectious agent can enter a new host (through broken skin, the respiratory tract, mucous membranes, and catheters and tubes)
6. **Susceptible host** can be *any* person (the most vulnerable of whom are receiving healthcare, are immunocompromised, or have invasive medical devices including lines, devices, and airways)



Hospital-Acquired Infections

- The term hospital infection, hospital-acquired infection, or nosocomial infection is applied to infections occurring in hospitalized patients who were neither infected nor were in incubation at the time of their admission to the hospital.
- Approximately, 5% of hospitalized patients experience a nosocomial infection. Nosocomial infection represents an important public health problem in developing countries, as in developed ones today, and as a major cause of high morbidity, mortality, and economic consequences in hospitalized patients.
- The impact of hospital-acquired infections is considerable—the patient may need longer hospital treatment, readmission, or even further surgery, increasing the time of absence from work and use of hospital and community resources.
- The recent trend of shorter hospital stays means that more patients with hospital-acquired infections are presenting to general practitioners in the community.
- In addition, as home administration of intravenous medications becomes increasingly common, cannula- associated infections, once confined to hospital patients, may present in the community.

Factors

- Hospital infections as a group differ from other community acquired infections, both in their patient profile and the severity and treatment of the disease caused by them. The factors influencing hospital-acquired infections are as follows:
  - Hospitalized patients
  - Hospital environment
  - Antibiotics resistance
  - Diagnostic or therapeutic procedures
  - Transfusion of blood

Sources of Infections

- The sources of hospital-acquired infection may be exogenous or endogenous. ***Exogenous infection*** is most important and occurs mostly from (*i*) another patient, (*ii*) a member of the medical and paramedical staff harboring the pathogens, or (*iii*) from the environment.
- ***Endogenous infection*** is due to microbes present in the patient's own flora present in the body.

Transmission of Infections

- Many organisms gain entry to the body through breaches or evasion of "first line" body defenses. Breaches in epithelial integrity (e.g., surgical wounds, intravascular cannulas, and drain tubes), loss of the washing action of body fluids (e.g., because of a urinary catheter), and interference with first-line respiratory defenses (e.g., by anesthesia and endotracheal intubation) are common precursors of hospital-acquired infections. Infections can be transmitted by following ways.
  - Air-borne transmission
  - Transmission by direct contact
  - Transmission by oral route
  - Transmission by parenteral route

Common hospital-acquired infections

S.No.	Nosocomial infection	Causative organisms
1	Urinary tract infection (catheterization related)	*Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Proteus* spp., *Pseudomonas* spp., *Enterococcus faecalis*, *Candida* spp., *Staphylococcus epidermidis*, and other coagulasenegative *staphylococci*
2	Wound infection (burns, postoperative, diabetic foot, etc.)	*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., coagulase-negative staphylococci, *Escherichia coli*, *Proteus* spp., *Enterococcus faecalis*, and *Streptococcus pyogenes*
3	Respiratory infection (ventilator-associated aspiration in comatose patients, etc.)	*Klebsiella* spp., *Enterobacter* spp., *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* spp., *Acinetobacter* spp., *Proteus* spp., and *Legionella pneumophila*
4	Gastrointestinal infection (food poisoning, transmission from infected staff, or patient, etc.)	*Salmonella* spp., *Shigella flexneri*, *Shigella dysenteriae*, *Shigella sonnei*, and *Escherichia coli* (in children and neonates)

Lab Diagnosis

- Diagnosis of hospital-acquired infections is made by routine bacteriological methods, such as direct demonstration of microorganisms in specimens by microscopy, isolation by culture, and testing bacterial isolates for their antibiotics sensitivity pattern.
- The specimens are usually sampled from possible sources of infections, such as environment, inanimate objects, water, air, food, hospital personnel, etc. The source of infection may be traced by performing phage typing, bacteriocin typing, biotyping, or molecular typing.

Prevention and Control

- Up to a third of hospital-acquired infections are preventable. The two main arms of prevention are stopping the development of antibiotic resistance and preventing the spread of resistant organisms between patients.

Components of a program to prevent hospital-acquired infection are many.

1. Compliance with hand washing protocols.
2. Use of aseptic technique for insertion of intravenous and urinary catheters.
3. Compliance with guidelines on antimicrobial use.
4. Proper patient care.
5. Short hospital stays.
6. Early removal of invasive devices.
7. Isolation of infectious patients.
8. Hospital infrastructure and policies.
9. Staff vaccination (e.g., hepatitis B, varicella-zoster, tuberculosis, and influenza).
10. Sharps policy.
11. Adequate sterilization and disinfection of surgical instruments and endoscopes.
12. Infection control program.
13. Active surveillance for hospital-acquired infection.

GLOSSARY

Adherence (adhesion, attachment): The process by which bacteria stick to the surfaces of host cells. After bacteria have entered the body, adherence is a major initial step in the infection process. The terms *adherence , adhesion* and *attachment* are often used interchangeably.

Carrier: A person or animal with asymptomatic infection that can be transmitted to another susceptible person or animal.

Infection: Multiplication of an infectious agent within the body. Multiplication of the bacteria that are part of the normal flora of the gastrointestinal tract, skin, and soon is generally not considered an infection; on the other hand, multiplication of pathogenic bacteria—even if the person is asymptomatic—is deemed an infection.

Invasion: The process whereby bacteria, animal parasites, fungi, and viruses enter host cells or tissues and spread in the body.

Microbiota: Microbial fl ora harbored by normal, healthy individuals.

Nonpathogen: A microorganism that does not cause disease; may be part of the normal microbiota.

Opportunistic pathogen: An agent capable of causing disease only when the host's resistance is impaired (ie, when the patient is "immunocompromised").

Pathogen: A microorganism capable of causing disease.

Pathogenicity: The ability of an infectious agent to cause disease. (See also virulence.)

Superantigens: Protein toxins that activate the immune system by binding to major histocompatibility complex (MHC) molecules and T-cell receptors (TCR) and stimulate large numbers of T cells to produce massive quantities of cytokines.

Toxigenicity: The ability of a microorganism to produce a toxin that contributes to the development of disease.

Virulence: The quantitative ability of an agent to cause disease. Virulent agents cause disease when introduced into the host in small numbers. Virulence involves adherence, persistence, invasion, and toxigenicity (see above).

Primary pathogens: It cause disease as a result of their presence or activity within the normal, healthy host and their intrinsic virulence (the severity of the disease they cause) is, in part, a necessary consequence of their need to reproduce and spread.

Endemic Diseases: The constant presence of diseases or infectious agents within a given geographic area or population group. It may also refer to the usual prevalence of a given disease with such area or group. It includes holoendemic and hyperendemic diseases.

Epidemic Diseases: It is the rapid spread of infectious disease to a large number of people in a given population within a short period of time, usually two weeks or less. For example, in meningococcal infections, an attack ratein excess of 15 cases per 100,000 people for two consecutive weeks is considered an epidemic.

Pandemic diseases: It is an epidemic of infectious disease that has spread across a large region; for instance multiple continents, or even worldwide.

A widespread endemic disease that is stable in terms of how many people are getting sick from it is not a pandemic. Further, flu pandemics generally exclude recurrences of seasonal flu. There have been a number of pandemics such as smallpox and tuberculosis. One of the most devastating pandemics was the Black Death, which killed over 75 million people in 1350. The most recent pandemics include the HIV pandemic as well as the 1918 and 2009 H1N1 pandemics.

UNIT-2
Bacterial infections

Organisms that are Gram stained:

- G+ cocci
- G- cocci
- G+ rods
- G- rods

Organisms not Gram stained:

- *Mycobacterium* – are acid fast
- *Mycoplasma* species – don't have cell wall so wont stain
- *Treponema* and *Leoptospira* species – spirochetes are too thin to be seen with Gram stain
- *Chlamydia* and *Rickettsia* species – stain well with Giemsa stain, but poorly with anything else.
- Acid fast bacteria can't be stained by Gram stain because their outer wall has a high concentration of lipids called mycolic acid that resists decoloration with alcohol!

Aerobic and Anaerobic Growth

- Oxygen provides metabolism and growth – oxygen acts as a hydrogen acceptor.
- The use of oxygen generates two toxic molecules: **Hydrogen Peroxide** (H2O2) and free radical **Superoxide (O3),**
- so bacteria need two enzymes in order to utilize oxygen: **Superoxide Dismutase** and **Catalase** – they remove the
- toxic compounds (only bacteria that have superoxide dismutase and catalase can use oxygen).
- The bacteria response to oxygen is important for diagnosis:

Anaerobe bacteria:

- Grow poorly in room temperature; they can only grow in an environment that contains less than 20% oxygen.
- Diseases of anaerobes are characterized by abscess, foul smelling discharge, gas in tissues and necrotic tissue.

Obligate aerobes:

- Need oxygen to grow because their ATP generation system is dependent on oxygen hydrogen acceptor.
- It grows better in *20% oxygen* of room air and don't grow at all under anaerobic conditions e.g. *M.tuberculosis*.

Obligate anaerobes:

- Cannot grow in the presence of oxygen because they lack either superoxide dismutase or catalase or both.
- Many of them use nitrogen rather than oxygen as terminal electron donor e.g. *Clostridium perfringes.*

Facultative bacteria:

- They use oxygen to generate energy via respiration IF oxygen is present, if not, they can utilize fermentation pathways to synthesize ATP e.g. E.Coli.

Aerotolerant organisms:

- Grow to some extent in air, but multiply faster in low oxygen concentration.

Microaerophilic organisms:

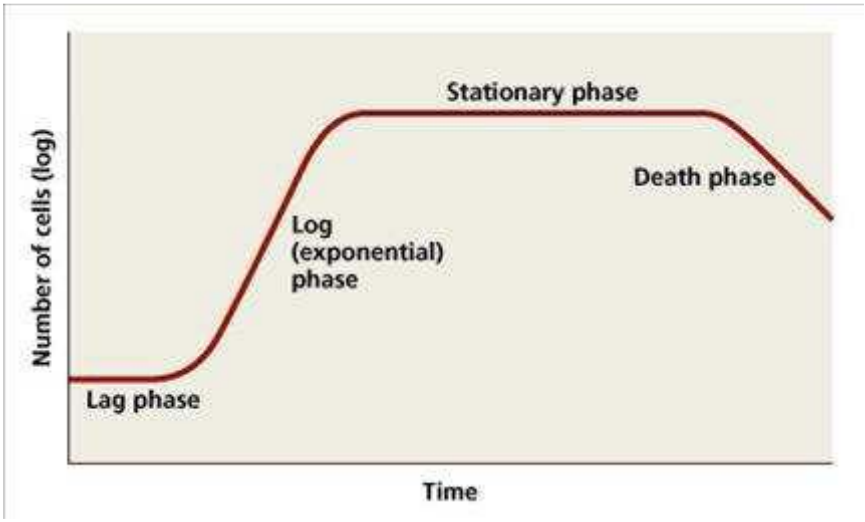
- Need reduced oxygen concentration (~5%) to grow optimally e.g. *Campylobacter jejuni*.

Fermentation

- Breakdown of sugars into pyruvic acid and then lactic acid, it is alternative way for bacteria to produce ATP in absence of oxygen.
- *N. gonorrhea* and *N. meningitidis* are distinguished base on fermentation of glucose or maltose.
- *E. coli* is differentiated from Salmonella and Shigella based on fermentation of lactose.

Bacterial growth

- Bacteria reproduce by **binary fission** _ one parent cell divides and gives rise to two progeny cells.
- The progeny cells undergo exponential growth (logarithmic growth) _ 24=16, so after 4 generations one bacterium produces 16 bacteria.
- The generation time varies between bacteria e.g.
- 20 min for E.Coli (100 after 3 hours)
- 24 hours for Mycobacterium tuberculosis
- The generation time also depends on presence of nutrients, temperature and pH.



- **Lag phase:** there is allot of metabolic activity, but no cell division – can last for a few minutes to several hours.
- **Log phase:** rapid cell division. B-lactam drugs (penicillin) act during this phase, since these drugs are effective when bacteria are making peptidoglycans (diving).
- **Stationary phase:** due to little nutrients or presence of too much toxins, the growth slows down until the number of cells produced balances the number of cells dying.
- **Death phase:** decline in viable bacteria.

Staphylococcus

Sub groups:

- S. aureus
- S. epidermidis
- S. saprophyticus

- It is important to know the difference between Staphylococci and Streptococci since most Staphylococci are penicillin G resistant. Their differentiation is done in 3 ways:

1. Gram stain:

- Staphylococci lie in **grapelike clusters**, seen with gram stain.

2. Catalase test:

- All Staphylococci have the enzyme catalase, they are **catalase+** (Streptococci don't!).
- Catalase breaks down H2O2 into O2 and H2O.

3. Culture:

- *S. aureus* (!) and certain Streptococci are **beta-hemolytic** _ they completely hemolyze RBCs on agar plate (hemoglobin is released).
- **Also note**: S. Aureus is the only one that is **coagulase positive**!!
- ¨ S. aureus will activate the enzyme *coagulase* _ will activate prothrombin _cause blood to clot. So when G+ cocci appear in clusters in culture, the lab will perform a *coagulase test*. If the test comes back as positive (gives *golden/yellow colonies*), it shows that it's S. Aureus. If it comes back as coagulase negative however with G+ cocci in clusters, it can be another strain of Staph. (*S. epidermidis* or *S. saprophyticus*).

S. aureus

Characteristics

- G+ cocci in clusters, *coagulase+, catalase+,* produces *β-lactamase* (so resistant to antibiotics).

Antigenic structure:

- Teichoic acid
- Protein A
- Capsule

Virulence factors:

- Coagulase
- Catalase
- β-lactamase

Exotoxins:

- **Exfoliatin**
- Toxic shock syndrome toxin (TSST)
- Enterotoxin
- Leucocidin _ kill WBC
- Hemolysins

Habitat

- Human skin and nose

Transmission

- Via hands (touching)

Pathogenesis

- *Staphylococci* are members of the normal microbiota of the human skin and respiratory and gastrointestinal tracts.
- Nasal carriage of *S aureus* occurs in 20–50% of humans. Staphylococci are also found regularly on clothing, bed linens, and other fomites in human environments.
- The pathogenic capacity of a given strain of *S aureus* is the combined effect of extracellular factors and toxins together with the invasive properties of the strain.
- At one end of the disease spectrum is staphylococcal food poisoning, attributable solely to the ingestion of preformed enterotoxin; at the other end are staphylococcal bacteremia and disseminated abscesses in all organs.
- Pathogenic, invasive *S aureus* produces coagulase and tends to produce a yellow pigment and to be hemolytic. Nonpathogenic, noninvasive staphylococci such as *S epidermidis* are coagulase negative and tend to be nonhemolytic.
- Such organisms rarely produce suppuration but may infect orthopedic or cardiovascular prostheses or cause disease in immunosuppressed persons. They may be refractory to treatment because of the formation of bioflims.
- *S aureus* with whom it shares phenotypic characteristics such as hemolysis and clumping factor.

Diseases

It is one of the most common causes of human infections!

- *Food poisoning*
- *Toxic shock syndrome (TSS)*
- *Scalded skin syndrome*
- *Pneumonia*
- *Impetigo* (skin infection)
- *Furunculous*
- *Osteomyelitis* (bone/bone marrow infection)
- Endocarditis
- Abscess of many organs

Toxin mediated Staphylococcal diseases:

- TSS
- Scalded skin syndrome
- Food poisoning

Clinical Findings

- A localized staphylococcal infection appears as a “pimple,” hair follicle infection, or abscess.
- Food poisoning caused to violent nausea, vomiting, and diarrhea; and rapid convalescence. There is no fever.
- Toxic shock syndrome is manifested by an abrupt onset of high fever, vomiting, diarrhea, myalgias, a scarlatiniform rash, and hypotension with cardiac and renal failure in the most severe cases.

Lab diagnosis

- Gram stained smear and culture _ gives yellow/golden colonies on blood agar (since they are coagulase+).
- Samples taken from spit, pus or blood.

Treatment

- ~99% of strains are penicillin G resistant – these can be treated with methicillin, some cephalosporins or vancomycin.
- **For methicillin resistant strains _ vancomycin.**

Prevention

- No vaccine available.
- Proper hygiene is important.
- Cefzoline is used to prevent surgical wound infections.

S. epidermidis

Characteristics

- G+ cocci in clusters, *coagulase-*, *catalase+*.

Virulence factors:

- Glycocalyx _ gives bacterium the ability to attach to artificial structures in body, stronger adherence.
- No exotoxins!

Diseases

- Endocarditis on prosthetic heart valves
- Prosthetic hip infection
- Intravenous catheters infection
- CSF shunt infection
- Neonatal sepsis
- It is the main cause of hospital acquired infections!

Lab diagnosis

- Gram stained smear and culture.
- On blood agar _ gives *white/grey pigmentation* with no hemolytic zone.

Treatment

- Vancomycin (it produces β-lactamase and is resistant to many antibiotics).

Prevention

- Proper hygiene.

S. saprophyticus

Characteristics

- G+ cocci in cluster, *coagulase-*, does not have protein A and produces no toxins.

Diseases

- May cause urinary tract infection in young women (since it may be a member of the normal flora of vagina).

Treatment

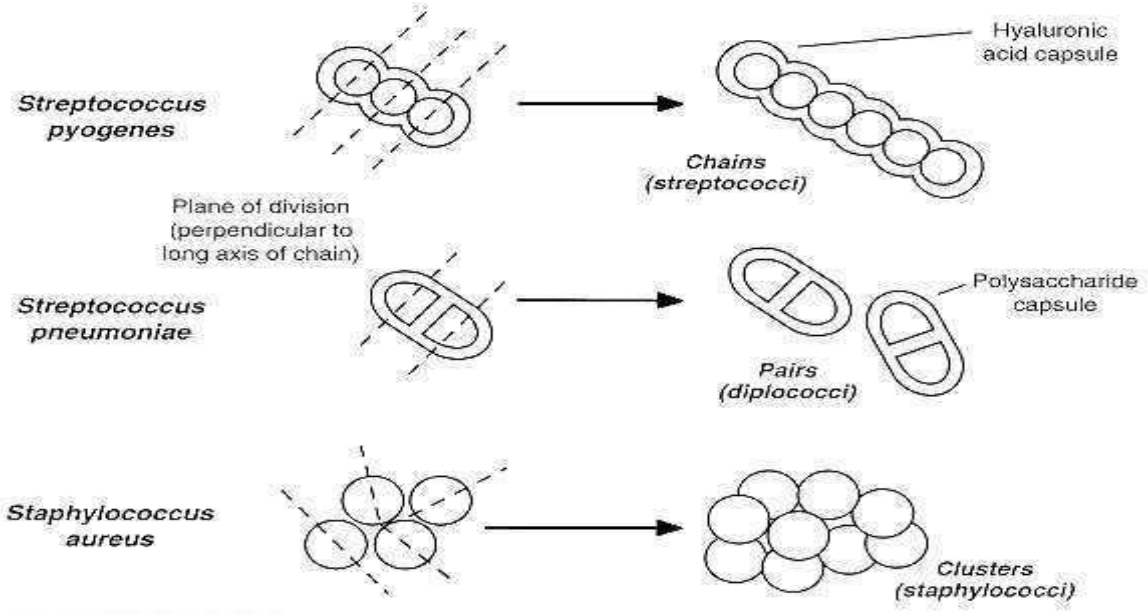
- Vancomycin (is antibiotic resistant).

Prevention

- Proper hygiene.

Streptococcus

- On gram stain, streptococci line up one after each other like strip of button candy.
- They are **catalase-.**
- Divided into 3 groups based on their specific hemolytic ability (after incubation on blood agar over night):
    - **Beta hemolytic streptococci** – completely lyse the RBCs _ leaving a clear zone of hemolysis around the colony.
    - **Alpha hemolytic streptococci** – partially lyse RBC _ leaving a greenish discoloration of culture medium surrounding the colony. The discolored area contains unlysed RBCs and green colored metabolites of hemoglobin.
    - **Gamma hemolytic streptococci** – unable to hemolyze RBCs, so they are non-hemolytic streptococci.
- They can also be classified based on the antigenic-characteristics of the C-carbohydrate (a carbohydrate found in the cell wall) – these antigens are called **Lancefield antigens** and are given letters **A – S**.
- There are over 30 species of streptococci; only 5 are significant human pathogens, 3 of these groups have Lancefield antigens: A, B, D. the other 2 don't (*S. pneumonia* and viridans group).
- The Lancefield group is determined by **coagglutination test** with specific antisera or by
- immunofluorescence.



Streptococcus pyogenes.

Characteristics

- G+ cocci in chain, beta-hemolytic, Lancefield group A and catalase-.

Antigenic structure:

- **C-polysaccharides _ determines the group specific antigens of s. pyogenes.**

- **M-protein _ determines the type specific antigens of s. pyogenes:**
  - It is the major virulence factor of *S. pyogenes*, those strains that lack M-protein are not virulent.
  - Immunity to infections is based on the presence of type specific antibodies to M-protein.
  - Since there are more than 80 types of M-protein, a person can have repeated infection with *S. pyogenes*.
- T-protein; has no relation to virulence.
- R-protein; has no relation to virulence.

Enzymes:
- ***Streptokinase***
- ***Streptodornase***
- ***Hyaluronidase***
- ***They all enhance the spread of bacterial infection in the body.***

Toxins:
- Hemolysins (streptolysins O, streptolysins S) _ responsible for beta-hemolysis on blood agar.
- Erythrogenig toxin _ causes scarlet fever.

Habitat
- Mainly in human throat and skin

Transmission
- Via respiratory droplets

Pathogenesis
- A variety of distinct disease processes are associated with *S pyogenes* infections. The infections can be divided into several categories.
- The portal of entry determines the principal clinical picture. In each case, however, there is a diffuse and rapidly spreading infection that involves the tissues and extends along lymphatic pathways with only minimal local suppuration. From the lymphatics, the infection can extend to the bloodstream.

Diseases

1. Pyogenic diseases (pus producing, fever inducing)
- **Cellulites, erysipelas, impetigo** (all inflammation of skin)
- **Fascitis** (inflammation of fascia)
- **Myositis** (inflammation of muscle)
- Pharyngitis and tonsillitis with fever, exudates and tender lymph nodes.

2. Toxogenic diseases (induced by exotoxins)
- **Scarlet fever**
  - **Caused by erythrogenic toxin _ is a superantigen causing capillary destruction.**
  - It gives skin rash and raspberry tongue.
  - **For treatment antitoxic antibodies are given which give life long immunity.**

3. Immunologic diseases/Post-streptococcal diseases

- **Glomerulonephritis**
  - Typically occurs 2-3 weeks after skin infection or pharyngitis
  - Clinical symptoms include: hypertension, edema of face and ankles and "smoky" urine.
  - The disease is based on antibody-antigen complex deposition n the glomerular basal membrane
  - **Type III Hypersensitivity.**
- **Rheumatic fever**
  - Occurs 1-4 weeks after pharyngitis with symptoms of fever, arthritis and carditis (carditis may lead to myocardial and valve damage).
  - Carditis is based on **Type II Hypersensitivity** (cytotoxic antibodies).
  - If the streptococcal infection is treated within 8 days of onset, rheumatic fever is usually
  - prevented.
  - After heart damage attack of rheumatic fever, re-infection must be prevented by **long-term prophylaxis**.
  - **The M-protein has the major pathogenic role in post-streptococcal diseases _ it induces hypersensitivity reactions**.

Lab diagnosis

- Culture on blood agar _ can observe very small colorless colonies with beta-hemolytic zones.
- Is sensitive to *bacitracin* and negative to *CAMP test.*
- Serological reaction: *ASO titer* is high in rheumatic fever (ASO titer shows if there has been a previous infection by *S. pyogenes*.

Treatment

- Penicillin G. Macrolides, such as erythromycin and clindamycin, have often been recommended for penicillin allergic patients and for patients with necrotizing fasciitis.

Prevention

- No vaccine, but penicillin is used in patients with rheumatic fever to prevent recurrent *S. pyogenes* pharyngitis _ this also prevents additional damage to heart valves.

Neisseria

- It is the only pathogenic G- cocci.
- They hang out in pairs, thus called diplococci.
- Each coccus is shaped like a kidney bean – they have their concave side facing each other.
- Two species cause human disease: *N. meningitides* and *N. gonorrhea*.
- Diagnosis involves Gram stain and culture of N. meningitides from blood, CSF or petechial scraping and of *N.gonorrhea* discharge from urethra.
- *Neisseria* grows best on blood agar that has been heated so that the agar turns brown _ chocolate agar. The classical medium for culturing *Neisseria* is called Thayer-Martin VCN media _ this is a chocolate agar with antibiotics which have been included to kill competitive bacteria:
  - V = Vancomycin _ kills G+ organisms

- C = Colistin (Polymyxin) _ kills all G- organisms except for *Neisseria*
- N = Nystatin _ kills fungi

- Therefore only *Neisseria* are able to grown on this culture medium.
- The addition of high concentration of CO2 further promotes the growth of *Neisseria*.
- In labs, the differentiation between the two species (N. meningitides and N. gonorrhea) is based on *N. meningitides*' ability to produce acid from maltose metabolism, which *N. gonorrhea* is not capable of doing.

N. meningitidis (Menigococcus)

Characteristics

- G-, "kidney bean" shaped diplococci, *oxidase positive*, *and ferments maltose*.
- It has a large *polysaccharide capsule* (it is one of that 3 encapsulated pyogenic bacteria, which all cause meningitis (*S. pneumonia* and *H. influenza* are the other two).
- Has 13 serological types base on capsular polysaccharide antigen (most diseases cause by type *B and Y*).

Virulence factors:

- Polysaccharide capsule _ antiphagocytic
- Lipopolysaccharide (LPS) _ endotoxin
- IgA protease _ cleaves secretory IgA so it can attach to mucosa

Endotoxins:

- LPS _ in cell wall, causes symptoms of septic shock; No exotoxins!

Habitat

- Human upper respiratory tract

Transmission

- Via respiratory droplets, site of entry is nasopharynx.

Pathogenesis and Clinical Findings

- The nasopharynx is the portal of entry. There, the organisms attach to epithelial cells with the aid of pili; they may form part of the transient flora without producing symptoms. From the nasopharynx, organisms may reach the bloodstream, producing bacteremia.
- Fulminant meningococcemia is more severe, with a high fever and a hemorrhagic rash; the patient may have disseminated intravascular coagulation and circulatory collapse.
- Meningitis is the most common complication of meningococcemia. It usually begins suddenly with an intense headache, vomiting, and stiff neck and progresses to coma within a few hours.
- During meningococcemia, there is perivascular infiltration and petechial hemorrhages. There may be interstitial myocarditis, arthritis, and skin lesions.

Diseases

- Meningococcemia – characterized by skin lesions.
- Acute (purulent) bacterial meningitis.

- After colonizing the upper resp. tract, the organism reaches meninges via blood stream. Deficiency in late complement component predisposes to recurrent meningococcal infections.

Lab diagnosis

- Fast, presumptive diagnosis: Gram or Methylene blue stain of CSF sediment and demonstration of bacterial capsular antigens by latex agglutination (from CSF).
- Oxidase positive colonies on chocolate agar, ferments maltose.

Treatment

- Penicillin G.

Prevention

- Chemoprophylaxis: Rifampin or ciprofloxacin.
- Vaccination: capsular polysaccharide (type A, C, Y and W135) – no vaccine against type B!

Mycobacterium leprae

Characteristics

- Aerobic, acid fast rod.
- Its *optimal growth* is at less that body temperature (*cooler temp*.), so lesions are on cooler parts of the body like *skin*, *nose* and *superficial nerves.*
- Can't grown on culture medium, may grow on *mouse foot pad* (specific test) or *armadillos*.

Habitat

- Humans are natural hosts (also found in armadillos, but not certain if they are source of infection for humans).

Transmission

- Most important mode of transmission is prolonged contact with patients with lepromatous form who discharge the bacterium in large numbers via *nasal secretions* and through *skin lesions*.
- Patients with lepromatous form are more likely to transmit the bacteria than those with tuberculoid form as they have a much higher bacteria count.
- The disease is found *world wide* with most cases in tropical areas of *Asia* and *Africa*.

Disease

Leprosy with two distinct forms:

1. Tuberculoid form:

- Hypopigmented macular skin lesions (due to cell mediated response to organism), thickened superficial nerves and anesthesia (loss of sensation) of skin occurs. The skin anesthesia results in burns and other traumas, which become superinfected.
- Resorption of bone leads to *loss of nose and fingertips*
- The *cell mediated* response is good in this form _ limits growth of bacteria.
- The *lepromin skin test will be positive*.

2. Lepromatous form:

- The *cell-mediated* response is *lost* so there are large number of organisms _ multiple skin lesions occur resulting in typical **leonine facies** (thickening and folding of skin due to infiltration of skin and nerves).
- The *lepromin test will be negative*.
- The bacterium lepricates intracellularly, typically within histiocytes, endothelial cells and Schwann cells of nerves.
- Incubation period is *several years.*

Lab diagnosis

- Lepromin skin test (positive in tuberculoid form and negative in lepromatous form).
- Culture and serological tests are not performed.

Treatment

- *Dapsone* combined with *Rifampin* for tuberculoid form.
- Clofazamine is added against lepromatous form or if the organism is resistant against Dapsone.
- Treatment lasts for *2 years.*

Prevention

- Dapsone for family members and those in close contact with infected people.
- No vaccine.

Leptospira interrogans

Characteristics

- Are tightly coiled, fine spirochetes that are not stained with dyes, but are seen with darkfield microscopy.
- They from on bacteriological media containing serum.
- No toxins and virulence factors known.

Habitat and Transmission
Reservoir: rodents, household animals (dog, swine etc).

Transmitted via *animals urine*

- The animal urine gets into fresh water and then Leptospira can enter the body through *cuts or other skin damage* or through mucous membranes (such as the inside of the mouth and nose). Once past the skin barrier, the bacteria enter the blood stream and rapidly spread throughout the body. The infection causes damage
- to the inner lining of blood vessels. The liver, kidneys, heart, lungs, central nervous system, and eyes may be affected.
- Leptospira Interrogans can survive for as long as six months outdoors under favorable conditions.
- Humans are *accidental hosts.*

Disease
Leptospirosis
Is a biphasic disease:

1st phase - bacteremic phase

- Lasts for about 3-7 days and presents as flue like symptoms (fever, chills etc).
- During this phase, bacteria can be found in the patient's blood and cerebrospinal fluid

2nd phase – immune phase

- Occurs either immediately after the bacteremic stage or after a one to three day symptom-free period.
- It can last up to one month.
- During this phase, symptoms are milder but meningitis, liver damage (jaundice) and renal failure are common.
- Bacteria can be isolated only from the urine during this second phase.
- Leptospirosis is mainly a disease of animals and can be a very serious problem in the livestock industry

Lab diagnosis

- Diagnosis made by serological testing for antibodies in patient's serum.

Treatment

- *Penicillin G.*

Prevention

- Doxycycline for short term exposure.
- Vaccination of domestic livestock and pets.
- Rat control.

Tuberculosis.

Most important species:

1. *M. tuberculosis* - Tuberculosis
2. *M. leprae* - Leprosy
3. *M. avium* - Intracellulare Atypical Mycobacterium
4. *M. kansaii* - They are opportunistic pathogens and can cause
5. *M. marinum* - tuberculosis in immunocompromised patients.
6. *M. fortuitum* – Chelonei Complex

Mycobacterium tuberculosis

Characteristics

- They are **obligate aerobes**.
- Rod shaped, don't form spores.
- Are **acid fast** _ ***their cell envelope contain high amount (60-70%) of complex lipids: mycolic acid and chord factor, so once the cells are stained (by carbol-fuchsin) they resist decoloration by acid-ethanol!!***
- They cannot be classified as G+ or G- (Gram stain not useful), have to use **Ziehl-Neelsen stain**!!
- Culture done on **Löwenstein-Jensen medium** – have to grow for 6-8 weeks.
- Their antigenic structure is *complex* – each type of mycobacterium has several proteins and polysaccharides.

- It grows very slowly, so drugs need to be present for a long time.

Virulence factors: Cord factor and **Mycolic acid** (long chain fatty acid).

Habitat

- **Human lungs** (humans are reservoirs).

Transmission

- Via respiratory droplets (coughing).

Pathogenesis

- Mycobacteria are emitted in droplets smaller than 25 µm in diameter when infected persons cough, sneeze, or speak. The droplets evaporate, leaving organisms that are small enough, when inhaled, to be deposited in alveoli. Inside the alveoli, the host's immune system responds by release of cytokines and lymphokines that stimulate monocytes and macrophages. Mycobacteria begin to multiply within macrophages. Some of the macrophages develop an enhanced ability to kill the organism, but others may be killed by the bacilli. One to 2 months after exposure, pathogenic lesions associated with infection appear in the lung. Resistance and hypersensitivity of the host greatly influence development of disease and the type of lesions that are seen.

Disease

- **Tuberculosis** _ fever, fatigue, night sweats, weight loss, chronic cough and hemoptysis.
- The initial site of infection is the lungs.
- The bacterium is facultative intracellular and may occur within cells or RES, especially monocytes and macrophages.
- M. tuberculosis causes lesions; their magnitude depends on the strength of the host's defense system.
- **The main immune defense against *M. tuberculosis* is activated macrophages**.

There are 2 types of lesions:

1. Exudative Lesions

- Consist of an acute inflammation of cells and occurs mainly in the lungs at initial site of infection.
- The parenchymal exudative lesion and the surrounding lymph nodes together are called a **Ghon-Complex.**
- Primary lesions – usually occur in the lower lobes (due to little oxygen content).
- Reactivation lesions – occur in apices (oxygenated). They can also occur in other well oxygenated organs like kidneys, brain and bones.
- It is during the exudative stage that the tuberculin test becomes positive!

2. Granulomatous Lesions

- These lesions consist of a central area of giant cells containing bacilli surrounding a zone of epitheloid cells.

- A tubercle is a granulomas surrounded by fibrous tissue that has undergone central caseous necrosis. Tubercles heal by fibrosis and calcification. They may break into a bronchus and form a cavity.
- When being infected for the first time, the bacteria spread via lymphatics and may reach blood stream and disseminate into many internal organs (military tuberculosis).
- Most cases of tuberculosis id due to reactivation in elderly and malnourished men.
- The risk of infection and disease is highest among socioeconomically disadvantaged people, who have poor housing and poor nutrition.

Koch's Phenomenon: shows the contrast between primary infection and reinfection in guinea pig. It is based on resistance and hypersensitivity induced by the first infection with bacterium. There will be and increased capacity of resistance against localized bacteria – it will slow down their growth/multiplication, limit their spread and reduce lymphatic dissemination.

Lab diagnosis

- Culture and staining as mentioned above from sputum, urine or CSF specimen.
    - **Acid fast staining (Ziehl-Neelsen)**
    - **Culture on selective media (Löwenstein-Jensen medium) liquid BACTEC medium**
    - **PCR amplification of bacterial DNA (with molecular probes)**
    - **Tuberculin Test (skin test) with PPD (Purified Protein Derivatives)**
- It induces *induration*, *edema* and *erythema* within in people who have previously been infected or been vaccinated against mycobacterium – the reaction is due to *delayed hypersensitivity*.
- The tuberculin test will become positive within 4-6 weeks after primary infection, not earlier.
- The test is positive if induration measures *10 mm* or more in diameter within *48-78 hours*.
- Negative test indicated the person has never been exposed.

Treatment

- Multiple drug therapy for 6-9 months with ***Isonaiazid (INH), Pyrazinamid, Rifampin (Ethambutol, Streptomycin)***
- In immunocompromised (AIDS) patients a fourth drug, *Ethambutol*, is added.
- Although therapy is given for months, the patient's sputum becomes noninfectious within 2-3 weeks.
- **Multiple drug therapy is used to prevent overgrowth of drug-resistant mutants during the long treatment period (if bacteria resistant to one drug emerge, they are most probably inhibited by the other drugs).**

Prevention

- Immunization with **BCG** (Bacillus Calmette Guerin) vaccine containing *live attenuated M. bovis* may prevent or

- limit disease but won't prevent infection!! It is given children in Europe and Asia
- Other preventive measures: eradication of tuberculosis in cattle, pasteurization of milk, fast and effective treatment of patients.

Gastrointestinal disorders

Gram negative rods related to enteric tract.

Categories of G-negative rods:
1. IN ENTERIC TRACT
a. Both inside and outside enteric tract
- *E.coli*
- *Salmonella*

b. Mainly inside enteric tract
- *Shigella*
- *Vibrio*
- *Campylobacter*
- *Helicobacter*

c. Only outside enteric tract
- *Klebsiella*
- *Enterobacter*
- *Serratia*
- *Proteus*
- *Providencia*
- *Morganella*
- *Pseudomonas*
- *Bacteroides*

2. RESPIRATORY TRACT
- *Haemophilus*
- *Legionella*
- *Bordetella*

3. ANIMAL SOURCE
- *Brucella*
- *Francisella*
- *Paterurella*
- *Yersinia*

- The entrobacteriacea (enterics) are a family of G-negative rods that are part of the *normal intestinal flora* and cause G.I. diseases.

Features common for all members:
1. They are *facultative anaerobes*
2. They all *ferment glucose* (some other sugars)
3. They are *oxidase negative* (non have cytochrome oxidase)
4. They *reduce nitrates to nitrites* as their energy generating process

All members contain *endotoxins* in the cell wall (since they are G-negative). In addition, several exotoxins are produces.

Antigenic classification

- The enterics for many groups based on cell surface structures that bind specific antibodies. They have 3 groups of antigens:

1. O-antigen

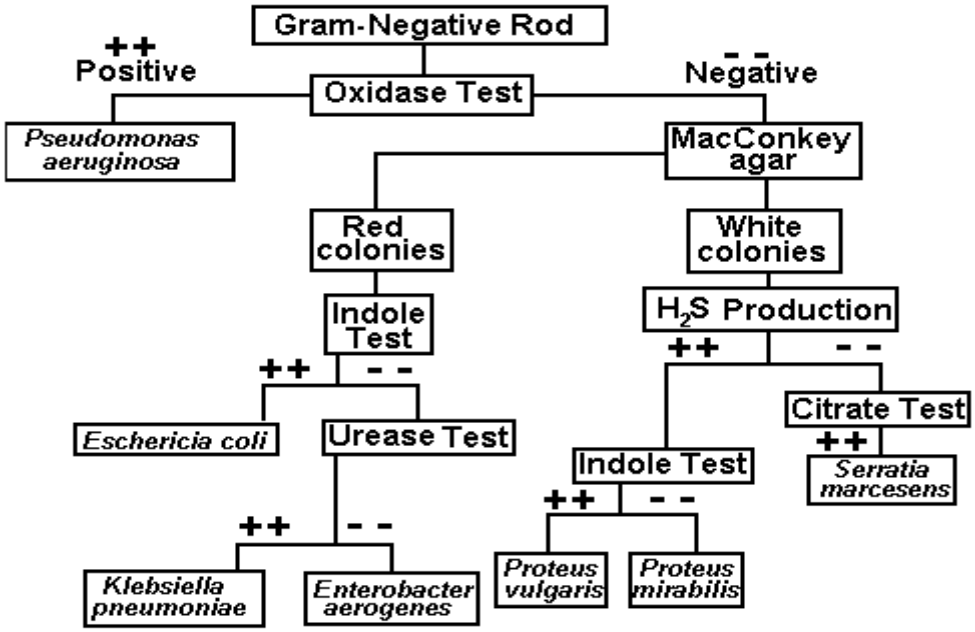
- Is the *cell wall* antigen. It is the outer most component of *lipopolysaccharide*. It is also the basis of serological typing of any enteric rods.

2. H-antigen

- Is on the *flagellar protein*. So only flagellated organisms have it!

3. K-antigen

- Is the *capsular polysaccharide antigen*. It is very prominent in heavily capsulated organisms. It is identified by the Quellung reaction (capsular swelling).



Laboratory diagnosis/Biochemical classification

Important properties that can be measure in the lab:

1. The ability to **ferment lactose** and turn it into *gas* – this can be visualized by including a dye that changes color with changes in pH. So on the specific media, the non-lactose fermenters form colorless colonies, while lactose fermenters form *colored colonies*!
2. The **production of H2O2** – this is based on its ability to *hydrolyze urea*, *liquefy gelatin* and *decarboxylate specific amino acids.*
3. Their **motility** – depends on presence of *flagella*, those who are very motile display swarms over the blood agar plate.

Some growth media do 2 things:

- They contain chemical that inhibit the growth of G+ bacteria which may have contaminated the samples.
- They have indicators which change color in the presence of lactose formation.

2 important media:

1) EMB agar (Eosin Methylene Blue agar)
- Methylene blue inhibits G+ bacteria.
- Colonies of lactose fermenters turn *deep purple/black* on this medium (e.g. E. Coli colonies get a *metallic green sheen* in this medium!).

2) McConkey agar
- *Bile salts* in the medium inhibit G+ bacteria.
- Lactose fermenters develop a *deep purple/pink coloration*.

Antibiotic therapy
- This varies, because so many of the organisms are highly antibiotic resistant due to production of **beta-lactamase** + other drug modifying enzymes.

***Salmonella* causing enteric fever.**
- Enteric fever is caused by: ***S. typhi*** & ***S. saprophyticus*****.**

Characteristics
- Facultative gram-negative rods, *motile* (peritrichous flagella), *non-lactose fermenting*, produce *H2S2*.

Antigenic structure:
- O-antigen
- H-antigen
- *Vi (virulence) antigen* _ is a capsular ("K") antigen

Virulence factors
- Endotoxin _ cause fever
- Vi-antigen _ antiphagocytic
- *Cytotoxin* _ is an enterotoxin
- Flagella
- Surface antigens for invasiveness
- No exotoxins!

Habitat
- Human *colon*, but also colonizes *vagina* and *urethra*.

Transmission
- Faecal oral route (contaminated food/water)

Pathogenesis and Diseases
- ***Salmonella typhi _ thyphoid fever***
- ***Salmonella paratyphi A, B, C _ enteric fever***
  - Incubation time is ~ 10-14 days.

- The bacteria will penetrate the intestinal wall and infect the regional lymphatic system some also enter blood stream and are carried to other parts of the body (liver and spleen).
- Patient gets *fever* (~ 40C) and *constipation* – may also see small *rose colored spots* on the stomach.
- Diarrhea starts during the 2nd or 3rd week of infection – during this time the bacteria are reinfecting the intestinal tract from the gallbladder and may cause necrosis of Peyer's patches.
- Symptoms will improve over time.
- Death rate ~ 2-10%. 20% relapse.
- Chronic carries can arise (3%) – the bacteria resides in the *gallbladder* (can tolerate high concentration of bile).
- Patient sheds the bacteria for years.

Symptoms

- Fever, malaise, headache, constipation, bradycardia, and myalgia occur. The fever rises to a high plateau, and the spleen and liver become enlarged. Rose spots, usually on the skin of the abdomen or chest, are seen briefly in rare cases. The white blood cell count is normal or low.
- In the pre-antibiotic era, the chief complications of enteric fever were intestinal hemorrhage and perforation, and the mortality rate was 10–15%. Treatment with antibiotics has reduced the mortality rate to less than 1%. The principal lesions are hyperplasia and necrosis of lymphoid tissue (eg, Peyer's patches); hepatitis; focal necrosis of the liver; and inflammation of the gallbladder, periosteum, lungs, and other organs.

Lab diagnosis

- During the first week, blood sample is used for culture, while during the second week of infection, stool sample is used _ see lactose fermenting colonies on EMB or McConkey agar.
- Blood sample for Gruber-Widal test _ detect rise in antibody titer in patients serum (antibodies for O- and H antigen).

Treatment

- Ampicillin – for chronic carriers.
- Ceftriaxone
- Ciprofloxacin

Prevention

Two vaccines:

- Acetone killed *S. typhi* – given intramuscularly.
- Live, attenuated *S. typhi* – taken orally.
- Public health measure (sewage disposal, chlorination of water etc).
- Personal hygiene

Vibrio cholerae.

- The Vibrionacea family includes *3 genera* that have clinical importance for humans:
    - **Vibrio**

- **Aeromonas**
- **Plesiomonas**

- Most of the infections caused by Vibrio species are enteric in nature and range from epidemic cholera to sporadic cases of diarrhea.

The Vibrio genus consist of
1. V. cholera
2. V. paraheamolyticus
3. V. mimicus

Vibrio cholera

Characteristics

- Short, comma shaped gram-negative rods, have a single polar flagellum (*monotrich*), are **oxidase positive** which distinguishes them from the other enterics!

Antigenic structure:

- 60 serogroups
- 3 serotypes
  - Ogawa
  - Inaba
  - Hikojima

Virulence factors:

- Flagellum _ adherence, penetration of intestinal mucosa
- Mucinase _ an enzyme that helps in penetration of mucous of small intestine
- Endotoxin
- Choleragen (cholera exotoxins)
- Subgroup A – responsible for *serological activity*
- Subgroup B – responsible for cellular *binding of toxin*.
- The mechanism of action of cholera toxin: cholera toxin activates adenylate cyclase enzymes in cells of intestinal mucosa leading to increased levels of intracellular cAMP and the secretion of large amounts of water, Na+, K+, Cl- and HCO3
- into lumen of small intestine.

Habitat

- Human colon

Transmission

- Faecal oral route

Pathogenesis

- Under natural conditions, *V cholerae* is pathogenic only for humans. A person with normal gastric acidity may have to ingest as many as 1010 or more *V cholerae* to become infected when the vehicle is water because the organisms are susceptible to acid. When the vehicle is food, as few as 10^2–10^4 organisms are necessary because of the buffering capacity of food.

- Any medication or condition that decreases stomach acidity makes a person more susceptible to infection with *V cholerae.* Cholera is not an invasive infection. The organisms do not reach the bloodstream but remain within the intestinal tract.
- Virulent *V cholerae* organisms attach to the microvilli of the brush border of epithelial cells. There they multiply and liberate cholera toxin and perhaps mucinases and endotoxin.

Diseases

Cholera

- Is an endemic disease in the Bengal region of India and Bangladesh and from here it has spread to the rest of the world. Since 1817 there has been

7 cholera pandemics, the most recent was in the 1960s.

Clinical findings

- Incubation period ~ 2-3 days, with an abrupt onset of diarrhea and vomiting.
- Fluid loss can be as high *as 1 liter/hour _ 15-20 liters/day*!
- The fluid is odorless "rice water".
- Hypovolemia, shock and metabolic acidosis may result in death. In untreated cases, fatality rate is 60%.

There are 2 types of carriers:

1. Convalescent carriers (= a person who harbors an infectious agent after recovery from a clinical attack of a disease).

2. Chronic carriers

- Usually over 50 years old
- They harbor the bacteria in the gallbladder.
- Their feces can contaminate water and food supplies – shed bacterial intermittently.

Lab diagnosis

- Isolation of bacteria on gram stained smear.
- Culture on TCBS agar (Thiosulfate, Citrate, Bile, Sucrose) – they tolerate alkaline conditions and are oxidase positive.
- Agglutination.

Treatment

- **Rapid intravenous or oral replacement of the hosts lost fluid and ions. Administration of isotonic maintenance solution should continue until diarrhea ceases. In severe cases _ administration of Tetracycline.**

Prevention

- Public health measures: sewage disposal, chlorination of water and proper food handling.
- Vaccine containing killed bacteria has limited effect, only 50% effective for 3-6 months.

Bacillary dysentery

Causative agent – *Shigella dysenteri*

Characteristics of *Shigella species*

- Facultative, G-negative rod, non-motile (no flagellum), no capsule, non-lactose fermenting (except for *S. Shigella*), don't produce H_2S; don't produce gas from glucose (except for *S. Flexneri*).

Antigenic structure:

- *Shigella* is divided into 4 serotypes based on ***O-antigen***.
  - **Serogroup A _ *S. Dysenteri*** with 12 serotypes
  - **Serogroup B _ *S. Flexneri*** with 6 serotypes
  - **Serogroup C _ *S. Bodyii*** with 18 serotypes
  - **Serogroup D _ *S. Sonneii*** with 1 serotype (it is biochemically distinct)
- Shigellas are the least resistant strain to physical and chemical agents. They can only tolerate low temperatures in adequate moisture _ they can survive for *6 months in water at* room temperature.

Virulence factors:

- Endotoxins
- Shigatoxin _ produced by group A *Shigella*. It is cytotoxic to enterocytes.
- Enterotoxin
- Invasive factor
- Capacity to replicate intracellularly.

Habitat

- Enteric tract of humans and animals.

Transmission

- Faecal-oral route
- Food/water bourne outbreaks may occur. The bacteria are transmitted from person to person by asymptomatic carriers via flies, fingers, food, feces.

Diseases

- **Enterocollitis** (Bacillary dysentery) = inflammation of small and large intestines.
- Incubation period ~ 1-2 days (short).
- The disease can be anything from an asymptomatic infection to severe bacillary dysentery with high fever, chills, abdominal cramps and *frequent bloody, mucoid stool*.
- Healthy adults can spontaneously cure in 2-7 days, but children, older people and malnourished individuals have a certain mortality rate due to dehydration and loss of electrolytes.
- After recovery most people shed the bacteria for a short period.
- There are some chronic carriers and disease may recur.
- Most dysentery are seen in children from 1-10 years of age.

- Shigella is the most effective pathogen _ ingestion of *100 (102) bacteria* may cause disease. Its efficiency is due to its resistance to stomach acid.
- Infections are almost always limited to G.I. tract.
- Shigella will penetrate the epithelial cells of colon _ multiply intracellularly _ leads to inflammation and cell death resulting in *bloody discharge* and *ulceration*.
- The cell death is performed by the toxins (neurotoxin, cytotoxin and enterotoxin); their mode of action is *inhibition of protein synthesis in host cells.*

Lab diagnosis

- Gram stained smear and culture.
- Fresh stool sample/rectal swab culture on EMB or McConkey's agar shows non-lactose fermenting colonies
- Methylene blue stain of fecal sample demonstrates if PMN leukocytes are presents or not.
- Slide agglutination test for determination of serogroup.

Treatment

- Rehydration
- In mild cases - no antibiotics needed
- In severe cases - ampicillin, amoxicillin, fluoroquinolones.

Prevention

- Chlorination of water, personal hygiene, proper food handling.
- No vaccine.

Syphilis.

Treponema Pallidum Caused to Syphilis

Characteristics

- Spirochete, G-negative, no toxins or virulence factors known.
- Are tiny Gram negative bacteria that look like *corkscrew.*
- They move in a spinning way with thin *endoflagella* called **axial filaments**, which lie between the outer membrane and peptidoglycan layer and wrap themselves around the length of the spirochetes.
- They are diagnostics problem because they can't be cultured in ordinary media – even though the all have G-negative cell membrane. They are *too small* to be seen in a microscope. So special procedures have to be used in order to screen for infection:
    1. **Darkfield microscopy**
    2. **Immunofluorescence**
    3. **Silver stains,**
    4. **Serological tests**

Habitat

- Human genital tract

Transmission

- Via sexual contact
- From mother to fetus across placenta.

Pathogenesis

- In 10–20% of cases, the primary lesion is intrarectal, perianal, or oral. It may be anywhere on the body. *T pallidum* can probably penetrate intact mucous membranes, or the organisms may enter through a break in the epidermis. Based on experiments in rabbits, as few as four to eight spirochetes may cause infection.
- Spirochetes multiply locally at the site of entry, and some spread to nearby lymph nodes and then reach the bloodstream. Within 2–10 weeks after infection, a papule develops at the site of infection and breaks down to form an ulcer with a clean, hard base ("hard chancre"). The inflammation is characterized by a predominance of lymphocytes and plasma cells.

Disease

- **Syphilis**
- Organism multiplies at site of inoculation and then spreads widely via the bloodstream.
- Many features of syphilis are attributed to blood vessels, involvement causing *vasculitis*.

Syphilis has different stages with different symptoms:

1. Primary Syphilis:

- Non-tender ulcer (hard chancre) – heal spontaneously.

2. Secondary Syphilis:

- Maculopapular rash on skin and Condylomata Lata on mucous membrane – heal spontaneously.

3. Tertiary stage:

- Granulomas in bone, muscle, & skin (gummas), CNS involvement/inflammation, (tabes dorsalis, paralysis progressiva), cardiovascular lesions (aortidis, aorta aneurysm).

Lab diagnosis

- Dark-field microscopy or immunofluorescence.
- Serology tests are important:
- Non-treponemal antibody test (VDRL or RPR) with Cardiolipin as antigen.
- Specific treponemal antibody test (FTA-ABS) with Treponemal Pallidum antigen.

Two antibodies which may indicate syphilis and test demonstrating them are:

1. Regin (nonspecific antibody), demonstrated with:

- RPR or VDRL (flocculation test).

2. Immobilisin (non specific antibody), demonstrated with

- TPHA (T. Pallidum hemagglutination,
- FTA-ABS (fluorescent treponemal assay with antibody absorption)

- TPI (T. Pallidum immobilization test)
- Advantages and disadvantages of the FTA-ABS syphilis serological test compared with VDRL test: specific (treponemal) test such as FTA-ABS are mores specific but they can not be used to follow the efficacy of treatment (because the specific antibodies persist even after effective eradication of bacteria)/

Treatment

- Penicillin (*T. Pallidum* grows slowly so drugs must be present for a long time).
- Other antibiotics (eg, tetracyclines or erythromycin) can occasionally be substituted.

Prevention

- Benzanthine penicillin given to those who have gotten into contact with infected people.
- No vaccine.

N. gonorrhea (Gonococcus)

Characteristics

- G-, kidney bean shaped diplococci, oxidase positive.

Virulence factors:

- Pilus _ attachment and antiphagocytic
- IgA protease
- Outer membrane proteins _ attachment + antigenic
- LOS (lipooligosaccharide)
- Note, no capsule!
- Endotoxin is present, but weaker than N. Meningitides, so less severe diseases occur.
- No exotoxins!

Habitat

- Human genital tract

Transmission

- Sexual contact, neonatal during delivery.

Pathogenesis

- Gonococci attack mucous membranes of the genitourinary tract, eye, rectum, and throat, producing acute suppuration that may lead to tissue invasion; this is followed by chronic inflammation and fibrosis.
- Men usually have urethritis, with yellow, creamy pus and painful urination. The process may extend to the epididymis. As suppuration subsides in untreated infection, fibrosis occurs, sometimes leading to urethral strictures. Urethral infection in men can be asymptomatic.
- In women, the primary infection is in the endocervix and extends to the urethra and vagina, giving rise to mucopurulent discharge. It may then progress to the

uterine tubes, causing salpingitis, fibrosis, and obliteration of the tubes. Infertility occurs in 20% of women with gonococcal salpingitis.
- Chronic gonococcal cervicitis and proctitis are often asymptomatic. Gonococcal bacteremia leads to skin lesions (especially hemorrhagic papules and pustules) on the hands, forearms, feet, and legs and to tenosynovitis and suppurative arthritis, usually of the knees, ankles, and wrists.

Diseases
- Gonorrhea
- Disseminated gonorrheal infection: arthritis, skin eruptions (endocarditis, meningitis).
- Neonatal conjunctivitis (Opthalmia Neonatrium)
- Pelvic inflammatory disease
- The organism invades mucous membrane and causes inflammation. Women during menstruation and pregnancy are prone to infection.
- After infection by *N. gonorrhea*, patient obtains partial immunity for short duration, but no protection from reinfection!

Lab diagnosis
- Observe oxidative positive colonies on Thayer-Martin medium (gonococci don't ferment maltose).
- **In acute *N. gonorrhea* infection; demonstration of bacteria (intracellular in PMNs) from urethral discharge by Gram or Methylene blue stain. PCR amplification of bacterial DNA.**

Treatment
- They are penicillin resistance due to plasmid encoded penicillinase.
- Ceftriaxone for uncomplicated cases.

Prevention
- No drug or vaccine
- Safe sex (condoms)
- **Silver acetate eye drops or erythromycin ointment to prevent neonatal conjunctivitis.**

Gram-positive spore forming rods:

Bacillus genus:
- *B. anthracis*
- *B. cereus*

Clostridium genus:
- *C. perfringes*
- *C. novyi*
- *C. septicum*
- *C. histolyticum*
- *C. tetani*
- *C. botulinum*
- *C. difficile*

- In spore forming bacteria, it is the **spores** that infect and make people sick!
- Both cause disease by releasing exotoxins

- *Bacillus* = aerobic
- *Clostridium* = anaerobic

ENDOSPORES

- Are metabolically dormant forms of bacteria that are resistant to hear, cold, drying and chemical agents.
- They are formed when there is a shortage of needed nutrients and can lie dormant for years!
- Surgical instruments are heated in autoclave –steam pressure at 121C for 15 min to kill spores.
- When spores are exposed to favorable nutrients, they become active again.

Tetanus.

Tetanus caused by *Clostridium tetani*

Characteristics

- Anaerobic, G+ rod (quite long), spore forming. Its spore is terminally located so the bacterium looks like a tennis racket. It has many peritrichous flagella

Antigenic structure:

- H-antigen _ 10 serotypes.

Virulence factor:

- Tetanus toxin (Retanaspasmin) _ is a protease that cleaves proteins involved in release of neurotransmitters.
- Retanaspasmin is a heat labile protein that can be inactivated by heating for 20 min and 60C. Tetanus toxin is one of the most poisonous substances known (only Botulinum toxin and *Shigella* dysentery toxin are comparable substances in toxicity).
- The mode of action of toxin: it blocks the release of inhibitory neurotransmitters (glycine, acetylcholineesterase, GABA) from spinal neurons. So excitatory neurons are unopposed and extreme muscle spas (tetanus, spastic paralysis, "lock jaw", "rhisus sardonicus") results. Muscle spasm can give respiratory difficulties.

Habitat

- Soil. Spores are present in G.I of humans and animals (horses).

Transmission

- The organism enters through skin wounds like simple puncture wounds from nails, splinters or thorns.

Pathogenesis

- In wounds, the oxidation reduction must be low _ this leads to tissue necrosis. After germination of spores, toxin is elaborated and gains entrance to CNS.

Disease

Tetanus

- Incubation period ~4-6 days.
- Symptoms of tetanus are spastic paralysis: meaning muscle spasm like lockjaw (trismus), rhisus sardonicus (grimace of the face), opistothonus (spasm of back) and respiratory paralysis. Sometimes the spasms can be so severe that hey cause bone fracture. The disease takes several weeks and death occurs due tone of the spasms.
- The poor prognosis is due to the short incubation period between injury and starting of seizure – there is rapid development from muscle spasm to tetanospasm.
- The worst form of tetanus, *tetanus neonatum*, is a very important cause of morbidity and mortality in developing countries. It results from cutting the umbilical cord with unsterile instruments or due to improper care of umbilical stump.

Lab diagnosis

- Primarily a clinical diagnosis (spasm etc).
- Microscopic (characteristic spore-forming drumsticks/tennis racket).
- Culture (anaerobic).

Treatment

- Hyperimmune human globulin (human antitoxin).
- Large doses of penicillin, tetracycline and clindamycine.
- Curare like agents to complete paralyze patient's muscle (stops spasm).
- Toxoids vaccine (formaldehyde treated), usually given to kids in combination with diphteria toxoids and petrussis vaccine.

Prevention

- Tetanus is almost always preventable with **DTaP** (tetanus and diphteria toxoids and killed petrussis).
- Wound should be cleaned and debrid, tetanus toxoids given, tetanus immunoglobulin (TETIG) given to previously
- unvaccinated patients and in case of heavily contaminated wound, penicillin may be added prophylactically.
- (This is all to prevent tetanus development in someone who is possibly contaminated with *C. tetanus* spores).

Clostridia causing gas gangrene.

C. perfringes (the most important one), ***C. novyi, C. septicum*** and ***C. histolyticum***.

C. perfringes

Characteristics

- G+, anaerobic, spore forming rod, has capsule but no flagella.
- Spore is subterminal and oval.

Antigen structure:

- A (found in intestinal tract of humans and animals), B, C, D and E exotoxin types.

Virulence factors:

- Hemolysin (alpha-toxin)
- Lecithinase
- Exotoxin
- Enterotoxin (is a superantigen) _ food poisoning.

Habitat

- Soil and human colon

Transmission

- Gas gangrene is due to contamination of wound with soil or feces (e.g. during war, car accident).
- Food poisoning is transmitted by ingesting contaminated food.

Diseases

Gas gangrene (myonecrosis) and food poisoning.

- Gas gangrene (caused by Type A) in wounds is due to germination of spores under anaerobic conditions and the production of several cytotoxic factors _ alpha toxin and lecithinase which cleave the cell membrane.
- The gas in tissue (CO2 and H2) is produced by bacterial anaerobic metabolism and production of toxins _ muscles get destroyed by the toxins resulting in *pain*, *edema* and *gas accumulation* (**crepitation**) _ circulation gets impaired decreasing the oxidation-reduction potential and pH, and this provides a new area within the muscle suitable for the growth of bacteria. So the disease progresses by moving into new areas in the muscle with the help of the destructive toxins.
- The incubation time from the time of injury until the appearance of symptoms is 12-24 hours.
- Hemolysis and jaundice is common + blood-tinged exudates followed by shock. Death occurs rapidly in untreated cases.

***C. perfringes* Type A**

- Also causes *food poisoning* by producing *θ-toxin* (enterotoxin).
- ~8-24 hours after ingestion of contaminated food (especially meat), the patient develops abdominal pain and diarrhea. Nausea may occur but vomiting is uncommon!
- Symptoms last for ~ 12-18 hours and recovery is usually complete.

***C. perfringes* Type C**

- Causes *enteritis necrotizans* _ is more severe than food poisoning.
- Incubation period is ~ 24hours with a sudden onset of severe abdominal pain, diarrhea and bloody stool _ can be fatal.
- Peripheral circulation can collapse or patient may develop intestinal obstruction or peritonitis.

Lab diagnosis

- Direct Gram stained smear.

- Culture in anaerobic conditions (spores not usually seen in clinical samples, however the organism will be growing if the nutrients area not restricted).
- Production of lecithinase is detected on egg yolk agar and is identified by enzyme inhibition with specific antiserum.
- Detection of toxin production in gas gangrene.

Treatment

- Removing all necrotic tissue + cleaning thoroughly the wounds in case of gas gangrene.
- Antitoxin therapy in gas gangrene.
- Hyperbaric therapy in gas gangrene.
- Antibiotic therapy with penicillin.
- Only symptomatic treatment in case of food poisoning.

Prevention

- Early and extensive wound debridement, cleansing, removal of necrotic tissue and prophylactic antibiotics in gas gangrene.
- In food poisoning – food should be adequately cooked.

***Escherichia Coli* (*E. Coli*)**

Characteristics

- G-negative rod, facultative anaerobe, ferments lactose, *motile* with peritrichous flagella.

Antigenic structure

- 150 O antigens
- 50 H antigens
- 9 K antigens

Virulence factors:

- Endotoxin (LPS) _ septic shock
- Enterotoxins:
    1. **Heat Labile (HL)** _ stimulates adenylate cyclase_increase cAMP_cause outflow of chloride ions and water_result in *diarrhea*.
    2. **Heat Stable (HS)** _ stimulate guanylate cyclase_results in *diarrhea*.
- **Verotoxin** _ produced by E. Coli with ***0157:H7 serotype*** _ ***causes bloody diarrhea and haemolytic uremic syndrome (HUS)***
- Capsule
- Pili

Habitat

- Human *colon*, but also colonizes *vagina* and *urethra*.

Transmission

- *Fecal-oral* route + during child birth.

Diseases

Intestinal diseases

1. ***Enteropathogenic E. Coli (EPEC)*** _ neonatal diarrhea
2. ***Enterotoxigenic E. Coli (ETEC)*** _ travelers' diarrhea
3. ***Enteroinvasive E. Coli (EIEC)*** _ bloody mucoid diarrhea
4. ***Enterohermorrhagive E. Coli (EHEC)*** _ hemorrhagic colitis and HUS, caused by ***E. Coli 0157:H7***.

Extraintestinal diseases

1. ***Urinary tract infection***

2. ***Neonatal meningitis and sepsis***
3. ***Nosocomial wound infection*** (hospital acquired infection)

Predisposing factors to UTI in women is the distance from anus to vagina/urethra, as well as having a short urethra _more common in younger age. Catheters also predispose for infection.

Lab diagnosis

- Gram stained smear and culture.
- Culture on EMB will show lactose fermenting colonies (*dark blue with green sheen*) + *swarming*.
- Specimens obtained from: urine, blood, stool or CSF.

Treatment

- Diarrhea: is self limiting, but rehydration might be needed.
- Neonatal meningitis and sepsis: ampicillin and 3rd generation cephalosporins.
- UTI: *ampicillin* and *sulfonamides*.

Prevention

- Limiting duration of catheter use.
- Prevent travelers' diarrhea by eating only cooked food and drinking boiled water.
- No vaccine.

Haemophilus influenzae

Characteristics

- Small gram-negative coccobacillus (elongated rod) with capsule.

Antigenic structure:

- Based on capsular polysaccharide.
- 6 serotypes (a-f).
- Antigen type **b** is *polyribose ribitolphosphate* _ swelling test.

Virulence factor:

- Capsule
- Lipooligosaccharide (LOS)
- IgA protease _ for attachment to mucosa.

Habitat

- Upper resp. tract.

Transmission

- Respiratory droplets.

Disease

- ***Purulent meningitis***: caused by capsule type b strain.
- High fever, headache, stiff neck. CSF is purulent.
- If untreated, fatality rate is 90%.
- ***Epiglottidis (obstructive laryngitis)***
- ***Otitis media and sinusitis:*** causes pain in affected area.
- Occurs mainly in young children.
- ***Pneumonia:*** mainly in elderly with chronic respiratory disease.

Lab diagnosis

- Gram stained smear on *chocolate agar* needs *X* (heme) and *V* (NAD) factor for growth.
- Can also be grown on blood agar with S. Aureus for satellite phenomenon.
- Latex agglutination _ to determine serotype.

Biochemical reactions.

Treatment

- ♣ Meningitis _ 3rd generation cephalosporins e.g. Ceftriaxone.
- ♣ Otitis media and sinusitis _ Ampicillin or Trimethoprim – Sulfamethoxazole.
- ♣ ~ 25% of the strains produce beta-lactamases.

Prevention

- ♣ *Vaccine containing capsular polysaccharide type b* is conjugated to carrier protein and given to children between ages of *2 and 15 months.*

Pseudomonas aeruginosa

Characteristics

- ♠ Aerobic, gram-negative rod, has 2-3 flagella (lophotricious).

Antigenic structure:

- ♠ O and H antigen.
- ♠ 15 serotypes (phage typing is important for differentiation between strains).

Virulence factors:

- ♠ Endotoxin _ fever and shock associated with sepsis
- ♠ Flagella
- ♠ Pili
- ♠ Glycocalyx
- ♠ *Exotoxin A* _ is identical to diphteria toxin; it inactivates EF2 _ stopping protein synthesis.
- ♠ *Exotoxin S* (function not clear)
- ♠ *Hemolysin* _ is a phospholipase C; it destroys pulmonary surfactant and attacks pulmonary tissue leading to necrosis.
- ♠ *Protease* _ important in corneal infection + it also degrades immunoglobulins, complement, coagulation factors and alpha-proteinase.
- ♠ Cytotoxin _ is a leukocidin.

Habitat

- ♠ *Pseudomonas* species are normally present in environment and can be isolated from skin, throat and stool of healthy people. They often colonize hospital food, sinks, taps, mops and respiratory equipment.

Transmission

- ♠ Transmission via aerosol, aspiration and faecal contamination.

Diseases

- ♠ ***Wound infection (burns)***
- ♠ ***UTI***
- ♠ ***Pneumonia (immunosupression)***
- ♠ ***Sepsis (immunosupression)***
- ♠ ***Otitis externa*** (known as swimmer's ears)
- ♠ Is the 3rd most common cause of nosocomial infection after S. Aureus and E. Coli.
- ♠ Is the leading cause of death in patients with cystic fibrosis, neoplastic disease and severe burns.
- ♠ Causes endocarditis and osteomyelitis in intravenous drug users.
- ♠ Corneal infections are caused due to infected contact lens fluid, cosmetics or due to trauma to eye.
- ♠ In patients with cystic fibrosis is causes chronic pneumonia, leading to lung destruction.
- ♠ UTI is manly due to predisposing factors like catheters.
- ♠ Mortality rate in immunocompromised patients with sepsis is ~80% and those with pneumonia ~70%.

Lab diagnosis

- ♠ Gram stained smear, culture and biochemical reactions.
- ♠ Can be isolated on almost any media, it is a very adaptable bacteria and can even grown in distilled water!
- ♠ On blood agar – see beta-hemolytic zones (due to hemolysis), oxidase positive and lactase negative. They also produce to pigments which may diffuse into agar*: blue pyocianin* and *yellow fluorescin.*

Treatment

- ♠ Certain penicillins: azlocillin, mezlocillin, piperacillin.
- ♠ Certain 3rd generation cephalosporins: ceftazidin, cefoperazon
- ♠ Aminoglycosides: gentamicin, Tobramycin, Amikacin.
- ♠ Carbapenems: imipenem, miropenem.

Prevention

- ♠ Disinfection of water related equipment in hospitals, hand wash and quick removal of catheters.
- ♠ No vaccine.

Bacillus genus

B. anthracis

Characteristics

- ♦ Aerobic, G+, spore forming rod.
- ♦ Has a capsule composed of *poly-D-glutamate* _ it is the only medically important organism that has a capsule made of amino acids instead of polysaccharides!

Antigen structure:

- ♦ Capsule polypeptide _ consist of G-glutamic acid (unique)
- ♦ Complex exotoxin, which has 3 components:
- ♦ Protective antigen _ attach to cells and mediates entry of the other two factors into cell.
- ♦ Lethal factor _ kills cells by inhibiting signal transduction proteins involved in cell division.
- ♦ Edema factor _ is an adenylate cyclase

Virulence factors:

- ♦ Capsule _ antiphagocytic
- ♦ Anthrax toxins (exotoxin).

Spores:

- ♦ Are formed in culture, soil and tissue of left over dead animals (not in tissue or blood of living animals!) – the spores are oval, centrally located and are extremely resistant to chemical and physical environment (temp. of 120C for 15 min is needed to kill them).

Habitat

- ♦ Soil

Transmission

Humans get infected in 3 ways:

1. **Cutanous route** _ through abrasions or cuts.
2. **Inhalation** _ spores from animal hair and wool.
3. **Ingestion** _ ingesting infected meat (rare).

Disease

- ♦ **Anthrax**.
    - o It is primarily a disease of herbivore animals, especially sheep and cattle. Humans accidentally encounter this disease in an agricultural setting. In

all 3 routes of infection – invasion of blood stream occurs followed by toxemia.

Cutanous anthrax:

- 95% of human cases.
- Disease starts 2-5 days after infection with a small papule that develops into a vesicle filled with dark bluish/black fluid.
- If vesicle ruptures, you will see a black eschar at the base of the papule with a local edema ring around it _ called **Malignant Pustule**. It is painless and usually found on hands, forearms or head.

Pulmonary infection:

- Via inhalation
- Known as "*wool-sorter's disease*".
- Starts with fever, malaise, myalgia and unproductive cough – within few days it becomes a very serious infection with *respiratory distress* and *cyanosis* _ death within 24 hours.

G.I. infection:

- Via ingestion.
- Is associated with nausea, vomiting, diarrhea and occasional loss of blood in stool.
- Associated with profound shock and death.

Lab diagnosis

- Gram stained smear and culture on blood agar with 5% CO2 (without CO2 the S _ R variation occurs).
- *B. anthracis* is **non-motile**, unlike *B. cereus*.
- Specimen for culture should be taken from a malignant pustule, sputum or blood.
- Fluorescent antibody test can also be used.

Treatment

- Penicillin G.
- Erythromycin for those allergic to penicillin.

Prevention

- Vaccine containing *purified protective antigen* – for people at high risk occupations.
- Active immunization (*living vaccine*) of herbivore animals to prevent spread to humans.
- Sterilize dead animal products so that soil doesn't get contaminated.
- Protective clothing.

Helicobacter pylori

Characteristics

- Ψ Curved, Gram-negative rods with lopotrich flagella.

Antigenic structure:

- Ψ It O and H antigens.

Virulence factor:

- Ψ Endotoxin _ LPS
- Ψ Flagella _ attachment
- Ψ *Urease* _ produces ammonia (alkaline pH) – neutralizes gastric juice and promotes with survival.
- Ψ Mucinase _ improves penetration.

Habitat

- Ψ Human stomach.
- Ψ No animal reservoir.

Transmission

- Ψ By ingestion – fecal oral route.

Diseases

- Ψ ***Gastritis***
- Ψ ***Peptic and duodenal ulcers***
- Ψ ***Gastric carcinoma***
- Ψ ***MALT lymphoma***
    - The bacteria attaches to gastric mucosa _synthesizes urease (produces ammonia) _ damages gastric mucosa. It also neutralizes the gastric pH in stomach, which allows the organism to survive.
    - **Symptoms are:** recurrent pain in upper abdominal region and frequent bleeding into G.I.

Lab diagnosis

- Ψ Biopsy specimen of gastric mucosa for culture on blood agar: microaerophilic (5% 02), oxidase+, resistant to nalidixiacid.
- Ψ **Urea-breath test** _ patients swallow urea labelled with an uncommon isotope, either radioactive carbon-14 or nonradioactive carbon-13. In the subsequent 10-30 minutes, the detection of isotopelabelled carbon dioxide in exhaled breath indicates that the urea was split; this indicates that urease (the enzyme that *H. pylori* uses to metabolize urea) is present in the stomach, and hence that *H. pylori* bacteria are present.

Treatment

- Ψ Amoxicillin and Metronidazole.
- Ψ Pepto-Bismol (Bismuth).

Prevention

- Ψ No vaccine or other preventive measure.

Corynebacterium diphteriae.

Characteristics

- ❖ Aerobic, non-spore forming, G+, club-shaped rods arranged in a **V or L shape** (look like *Chinese characters*).
- ❖ The rods have a *beaded* appearance. The beads consist of granules of polyphosphate called *Babes-Ernst granules* which stain *metachromatically*.
- ❖ Virulence factors: ***Diphteria toxin*** (exotoxin) – encoded by Lysogenic conversion
    - ŏ **It inhibits protein synthesis and peptide elongation in ribosomes by adding ADP-ribose to elongation factor 2**
    - ŏ **(EF-2).** The toxin has 2 components:
        - **Subunit A** _ ADP ribosylation activity.
        - **Subunit B** _ binds the toxin to cell surface receptors.

Habitat

- ❖ Human throat.

Transmission

- ❖ Via respiratory droplets.

Disease

- ❖ **Diphteria**
    - ŏ Toxin is absorbed into the mucous membrane and causes death of mucosal epithelium leading to formation of grayish, tough, adherent

pseudomembrane on tonsils, pharynx and larynx (local inflammation with exudates).

- ŏ The extensions of psuedomembranes into larynx and trachea can cause *airway obstruction*.
- ŏ Regional lymph nodes enlarge, fever and sore throat often occur.
- ŏ The toxin also produces nerve damage _ paralysis of eyes and extremities (myocarditis with *arrhythmias* and *circulatory collapse*). Recurrent *Laryngeal Never Parsy* can also be a complication (damage to recurrent laryngeal nerve leading to paralysis of larynx).
- ŏ *Cutanous edema* causes *ulcerating* skin lesions but without systemic symptoms.

Lab diagnosis

- ❖ Smears of throat swab should be stained with methylene blue or Neisser stain.
- ❖ Bacteria are cultured on Löffler's or tellurite (Clauberg) medium.
- ❖ Toxin production must be demonstrated by agar precipitation (ELEK-test).
- ❖ **Schick-test**: skin test which is base on irritation and local reaction of diluted toxin. If the patient has no antitoxin, the toxin will cause inflammation at the site of irritation ~4-7 days later. If *no inflammation* occurs, antitoxins are resent and the patient is immune.

Treatment

- ❖ **Antitoxin antibodies _ give life long immunity.**
- ❖ Penicillin and Erythromycin.

Prevention

- ❖ Toxoid vaccine (formaldehyde treated), usually given to children in combination with tetanus toxoid and petrussis vaccine (DTaP).

ATYPICAL MYCOBACTERIUM

- ✓ Called atypical because they differ from *M. tuberculosis* in various ways:
  1. Most important difference being that the ***reservoir for the atypical Mycobacterium is the environment (soil and water)***, while tuberculoid is found only in humans.
  2. The atypical are not transmitted from person to person.
  3. The atypical are opportunistic pathogens _ they can only cause disease when the host's resistance in low.
- ✓ They are subdivided into **SLOW** and **FAST** growers based on whether they *form colonies in more than or less than* **7 days** and if they have **pigment** production _ **Runyon Classification.**

1. PHOTOCHROMOGENES (slow growers, pigment production in light)

1. M. kansaii

- ✓ Causes lung disease resembling tuberculosis.

2. M. marinum

- ✓ Causes "swimming pool granulomas" _ ulcerating skin lesions at site of abrasion acquired in swimming pools or aquarium.

2. SCOTOCROMOGENES (slow growers, pigment production in the dark)

1. ***M. gordonae***

2. M. scrofulaceum

- ✓ Causes scrofula, manifested as swollen, nontender cervical lymph nodes (cervical adenitis). Usually seen in children.

3. NON CHROMOGENES (slow growers, no pigment production)

1. **M. Avium Intracellulare Complex (MAC)**
   - ✓ It causes TB like disease, especially in immunosupressed patients (like AIDS) patients, ~*25%* of *HIV* infected develop MAC.
   - ✓ MACs are resistant to first like of drugs (INH, Rifampin, and Pyrazinamid), so therapy should consist of new macrolides (Clarithromycin) + Ethambutol or Fluoroquinolones + Amikacin. Therapy has to last out life.

4. SAPROPHYTICUS (fast growers)

1. ***M. fortuitum***
   - ✓ Rarely causes human disease, but can cause infection of prostatic joint and catheters and also at site of skin punctures. Is resistant to most antituberculosis drugs.
2. ***M. smegmatis***

Rickettsia & Chlamydia

- Both are intracellular parasites, meaning they can only survive by establishing residence inside host cell. They need the host's ATP as an energy source for their own cellular activity.
- They are energy parasites, using cell membrane transport system that steals an ATP from the host's cell and spits out an ADP. Both of them have this ATP/ADP translocator, the difference is that Rickettsia can oxidize certain molecules and create ATP (via oxidative phosphorylation) while Chlamydia can't (has no mechanism for ATP production.
- It is impossible to culture Rickettsia and Chlamydia in non-living organism or non-living artificial media because the media don't produce ATP. **So since they are obligate intracellular bacteria they can only be cultured in experimental animals, embryonated eggs and cells culture** (McCoy cells).

Rickettsia Genus, *Coxiella Burnetii*.

- The family consists of 3 genera that infect humans: **Rickettsia**, **Rochalimaea** and **Coxiella**

Characteristics

- Small, G-negative, pleomorphic, rod-shaped to cocci.
- Rickettsiae are *obligate intracellular parasites* – adapted to intracellular multiplication in arthropods like flea, louse, tick and mite.
- They are all associated with the *reticuloendothelial cells* (RES) or *vascular endothelial cells* (except for Rochalimaea Quintana).
- They stain poorly with Gram, but can be visualized by using both **Giemsa** and **Machiavelli** method.
- Coxiella is smaller than other Rickettsia, there are two distinct types of Coxiella, designated **Large Cell Variants**
- **(LCV)** and **Small Cell Variants (SCV**) – they can be separated by gradient centrifugation.

Antigenic structure:

- Group specific
- Type specific

Virulence factors:

- Endotoxin
- Hemolysin (Phospholipase A) _ Rickettsia enters the endothelial lining of blood vessels with the help of phospholipase A.

- Resistance _ only C. Burnetii is very resistant to heat and drying, the others are not.

Culture

- **Yolk sac** or embryonated eggs are used, except for Rochalimaea, which can be cultured on cellfree media (blood agar).
- In the yolk sac the Coxiella undergoes a developmental cycle which involves formation of an *endospore-like body*.
- In addition they also undergo a **phase variation** – in nature Coxiella exist in phase I, but in the yolk sac the phase I organism is converted to phase II state.
- The LPSs of phase I and phase II antigens are different from LPSs of Gram negative bacteria.

Rickettsia has 2 biotypes:

1. Typhus group:

1. R. prowazekii
2. R. typhi
3. R. tsutsugamushi

2. Spotted fever group:

1. R. rickettsii
2. R. akari
3. R. canonii
4. R. australis (tick)
5. R. siberica (tick)

- For **Transmission** and specific **Diseases** see table next page!

Clinical findings

- Endothelial damage secondary to vasculitis (angiitis) is a common finding in Rickettsial infections, especially in Spotted Fever and Typhus.
- Occlusion of small vessels, microthrombi, microhemorrhages and in severe cases necrosis, shock and death may occur.
- Incubation period ~3-12 days.

Lab diagnosis

- Weil-Felix test (based on antigenic cross reactions between Rickettsial antigens and polysaccharide O-antigens from various strains of Proteus Vulgaris, OX2, OX19, OXK). The test is a tube agglutination test in which these proteus strains are used as antigens to demonstrate antibodies from patient's serum sample.
- The test is negative in Q-fever (C. burnetti) and in Trench Fever (R. quintana).
- Type specific complement fixation test.
- Indirect immunofluorescence.
- ELISA.

Treatment

- Tetracycline and Chloramphenicol.
- Without treatment, mortality is 20%.

Prevention

- Protective clothing and insect repellent
- Fast removal of ticks
- Pasteurization of milk kills Coxiella
- A Typhus vaccine containing formalin-killed R. Prowasekii is available.

Chlamydia Genus

Chlamydia has 3 genera:

1. C. trachomatis
2. C. psittaci

3. C. pneumoniae

Characteristics

- *Obligate intracellular* parasites.
- Cell wall is similar to G-negative, but has *high lipid content*.
- It has not N-acetyl-muramic acid _ so lysozyme is not effective.

Antigenic structure:

- Group specific antigens _ LPS with keto-deoxy-octonic acid
- Type specific _ outer membrane proteins.

Culture

- Not possible to culture on agar media, only in *yolk sac* of embryonated eggs or tissue culture cell lines (*McCoy cells*)

The developmental cycle is special:

- The infectious particle is called **"elementary body" (EB)** _ it attaches to the host cell _ gets engulfed by the host cell and then reorganized into larger **"reticular body" (RB)** (they are non infectious!) _ these will grow in size and divide repeatedly by binary fission forming inclusions in host cell in order to infect new cells. The cycle takes ~24-48 hours.
  - **EB** stains **purple** with Giemsa stain while the *host cell cytoplasm* stains *blue*.
  - **RB** stains **blue** with Giemsa stain.
  - When staining with *Lugo*, the **inclusions** of C. Trachomatis will stain **brown** due to the glycogen mix **(Halberstadter- Prowazek inclusion)**

Main phases of the developmental cycle:

1. Attachment and penetration of EB
2. Transition of metabolically inert EB into metabolically active RB
3. Growth and division of RB and produce of many progeny RB
4. Maturation of many noninfectious RB into infectious EB
5. Release of EB from the host cell

Lab diagnosis

- Specimen is scraping of epithelial cells from urethra or cervix _ perform IF or cell culture.
- **Frei test** _ skin test based on delayed hypersensitivity.

Treatment

- Antibacterial drug against Chlamydia infections: Tetracycline, Erythromycin and Azithromycin.

Prevention

- Early diagnosis and treatment of infected person.
- Safe sex.
- Prevention of neonatal eye disease depends on diagnosis and treatment of pregnant women and sex partners.

ZOONOTIC INFECTION

- Zoonoses from (Greek: *zoon* "animal" and *nosos* "ailment") are infectious diseases of animals (usually vertebrates), that can naturally be transmitted to humans.
- Major modern diseases such as Ebola virus disease and influenza are zoonoses. Zoonoses can be caused by a range of disease pathogens such as viruses, bacteria, fungi and parasites.

Causes

- Zoonotic transmission can occur in any context in which there is companionistic (pets), economic (farming, etc.), predatory (hunting, butchering or consuming wild game) or research contact with or consumption of non-human animals, non-human animal products, or non-human animal derivatives (vaccines, etc.).
  - Contamination of food or water supply
  - Farming, ranching and non-human animal husbandry
  - Wild animal attacks
  - Insect vectors
  - Pets
  - Exhibition
  - Hunting and bushmeat
  - Zoophilia
  - Secondary transmission

LIST OF ZOONOTIC DISEASES

Disease	Pathogen	Animals involved	Mode of transmission
Haemorrhagic fevers	Ebolavirus	Chimpanzees, gorillas, fruit bats, monkeys, forest antelope and porcupines	body fluids, organs
Rabies	Rabies virus	Dogs, bats, monkeys, raccoons, foxes, skunks, cattle, wolves, coyotes, mongooses and cats	Saliva by biting, or through scratches from an infected animal.
Anthrax	*Bacillus anthracis*	Cattle, sheep, goats, camels, horses, and pigs	by ingestion, inhalation or skin contact of spores
Influenza	Influenza virus	Horses, pigs, domestic and wild birds, wild aquatic mammals	Respiratory droplets
Leptospirosis	*Leptospira interrogans*	Rats, mice, dogs,	Direct or indirect contact with urine of infected animals
Bovine spongiform encephalopathy (BSE)	Prions	Cattle	Eating meat or bone meal of made from infected animals.
Brucellosis	*Brucella*	Cattle, Goats	Infected milk or meat
Foodborne illnesses	*Campylobacter, Escherchia coli, Salmonella, Shigella species and Trichinella*	Cattle, poultry	Food made from infected animals

Haemorrhagic fevers, Lassa fever, Crimean-Congo haemorrhagic fever(CCHF)	Commonly Viruses	Camels, hares, hedgehogs, cattle, sheep, goats, horses and swine	Direct contact with infected animals
Bovine Tuberculosis	*Mycobacterium bovis*	Cattle, deer, llamas, pigs, domestic cats, wild carnivores and omnivores	Milk, exhaled air, sputum, urine, faeces and pus from infected animals
Cysticercosis & Taeniasis	*Taenia solium*	Pigs	Eating water or food contaminated with the tapeworm eggs(Cysticercosis)
Echinococcosis	Echinococcus	Dogs, foxes, wolves, sheep, and rodents	Eating organs contaminated with the cysts of the worm
African sleeping sickness	Trypanosoma bruceirhodesiense	Range of wild animals and domestic livestock	Bite of the tsetse fly

Use in vaccines

The first vaccine against smallpox by Edward Jenner in 1800 was by infection of a zoonotic bovine virus which caused a disease called cowpox. Jenner had noticed that milkmaids were resistant to smallpox. Milkmaids contracted a milder version of the disease from infected cows that conferred cross immunity to the human disease. Jenner abstracted an infectious preparation of 'cowpox' and subsequently used it to inoculate persons against smallpox. As a result, smallpox has been eradicated globally, and mass vaccination against this disease ceased in 1981.

UNIT-3
Fungal Infections.

- Fungi differ from bacteria in cell structure as they are **eukaryotes**, while bacteria are prokaryotes.
- They lack chlorophyll – so can't make energy through photosynthesis.
- There are ~ 1000 known species, only ~ 100 cause disease in humans and animals.

FUNGAL CELL STRUCTURE

Fungal cell membrane:

- Is the inner most layer that surrounds the fungal cytoplasm (bilayered).
- Contains **ergosterol**, whereas human cell membrane contains cholesterol.
- Antibiotics like Amphotericin B and Nystatin bind to ergosterol and make holes in the fungal cell membrane, while azole drugs like Ketokonazole inhibit ergosterol synthesis.

Fungal cell wall:

- Surrounds cell membrane.
- Consist mainly of **chitin**, which is a polysaccharide (not peptidoglycan as in bacteria) – thus fungi are insensitive to antibiotics like penicillin, which inhibit peptidoglycan synthesis.
- Also contain polysaccharides like **beta-glucan** – which is the site of action of antifungal drug Caspofungin.

Fungal capsule:

- Is a polysaccharide coat that surrounds cell wall.
- Antiphagocytic.
- Only **Cryptococcus neoformans** has it!!
- Can be seen by staining with Indian ink stain.

There are two morphologically different fungi:

1. Yeast

- They grow as single cells (**unicellular**)
- Oval or round in shape
- Reproduce by **asexual budding** (they reproduce slower than bacteria)
- If the buds don't separate, they will form long chains called **pseudohypae**.

2. Molds

- For **multicellular** colonies
- They grown as long filaments (**hyphae**) and for a mat called **mycelium**
- Some hyphae form transverse walls _ **septate hyphae**, while others
- Don't _ **nonseptate hyphae**.
- Nonseptated hyphae are multinucleated (*coencytic*)
- ***Most fungi are thermally dimorphic _ meaning they can form different***
- ***structures at different temperatures:***
- ***Exist as mold in the environment and ambient temperature (25C).***
- ***Exist as yeast in human tissue at body temperature (37C).***
- Most fungi are **obligate aerobes**, some are facultative anaerobes, but none are obligate anaerobes!
- All fungi need performed *organic source* like *carbon* for survival – that's why they are frequently associated with decaying matter _ this is why the natural habitat

for most fungi is the environment like for saprophytes (except for Candida Albicans, which is part of normal human flora).

Some fungi reproduce *sexually* by mating and form sexual spores:

1) **Zygospores** _ single large spores with thick walls
2) **Ascospores** _ formed in a sac called ascus
3) **Basidiospores** _ formed externally on the tip of a fungi

- Fungi that don't form spores are termed "*imperfect*" are classified as **Fungi Imperfecti**.
- Most medically important fungi propagate asexually by forming **conidia** (**microconidia**-small, **macroconidia**-large), these are called **asexual spores**. They are formed either from the sides or ends of specialized structures.
- The *shape*, *color* and *arrangement* of conidia help in identification of fungi.

Some important conidia:

1. Arthospores:

- Results from fragmentation of ends of hyphae.
- Is the mode of transmission of *Coccidioides*.

2. Chlamydospores:

- Develop inside hyphae, either terminal or central.
- They are rounded, thick walled and quite resistant e.g. *Candida*.

3. Blastospores:

- Formed by budding, if the buds don't separate they form pseudohypae (yeast) e.g. *Candida*.

4. Sporangiospores:

- Formed within a sac (sporangium) at the end of a stalk.
- Done by molds like *Mucor*.

So fungi are classified based on:

1. **Sexual reproduction** (sexual or imperfect)
2. **Morphological characteristics** (yeast, mold etc)
3. **Classification of spores** (artho-, blasto-, etc.)

Key points:

Yeast: Yeasts are round or oval unicellular fungi that reproduce by asexual budding. On culture medium, such as Sabouraud's dextrose agar (SDA), they produce creamy mucoid colonies. Example: *Cryptococcus neoformans.*

Yeast-like fungi: These are the yeasts with pseudohyphae. Example: *Candida albicans.*

Molds: Molds grow as long filaments called *hyphae*. They usually measure 2–10 _m in width. Some hyphae form transverse walls and hence they are called septate hyphae, whereas others do not produce walls, hence are called nonseptate hyphae. Nonseptate hyphae are multinucleated. The hyphae on their continuous growth form a mat known as *mycelium*. The part of the mycelium that projects above the surface in culture medium is called aerial mycelium. Examples include *Aspergillus*, *Penicillium*, *Rhizopus*, etc.

Dimorphic fungi: Many of medically important fungi are dimorphic. They exist as hyphal/mycelial forms in the soil and in the cultures at 22–25°C. They occur as yeasts or other structures in human tissue and in the culture at 37°C. Examples include *Coccidioides immitis*, *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Sporothrix schenckii.*

The Major Mycoses and Causative Fungi

Fungal infections, depending on the tissues that are initially colonized, can be classified into three major groups as follows:

Superficial mycoses: These are surface infections of the skin, affecting the outermost layers of skin, hair, and mucosa.

Cutaneous mycoses: These are infections of the skin involving the epidermis and its integuments, the hair, and nails.

Subcutaneous mycoses: These are infections of the dermis, subcutaneous tissue, muscle, and fascia.

S.No.	Category	Mycosis	Causative Fungal Agents
1	Superficial	Pityriasis versicolor	*Malassezia* species
		Tinea nigra	*Hortaea werneckii*
		White piedra	*Trichosporon* species
		Black piedra	*Piedraia hortae*
2	Cutaneous	Dermatophytosis	*Microsporum* species, *Trichophyton* species, and *Epidermophyton floccosum*
		Candidiasis of skin, mucosa, or nails	*Candida albicans* and other *Candida* species
3	Subcutaneous	Sporotrichosis	*Sporothrix schenckii*
		Chromoblastomycosis	*Phialophora verrucosa, Fonsecaea pedrosoi,* and others
		Mycetoma	*Pseudallescheria boydii, Madurella mycetomatis,* and others
		Phaeohyphomycosis	*Exophiala, Bipolaris, Exserohilum,* and other dematiaceous molds
4	systemic	Coccidioidomycosis	*Coccidioides posadasii* and *Coccidioides immitis*
		Histoplasmosis	*Histoplasma capsulatum*
		Blastomycosis	*Blastomyces dermatitidis*
		Paracoccidioidomycosis	*Paracoccidioides brasiliensis*
5	Opportunistic	Systemic candidiasis	*Candida albicans* and many other *Candida* species
		Cryptococcosis	*Cryptococcus neoformans* and *Cryptococcus gattii*
		Aspergillosis	*Aspergillus fumigatus* and other *Aspergillus* species
		Hyalohyphomycosis	Species of *Fusarium*, *Paecilomyces*, *Trichosporon*, and other hyaline molds
		Phaeohyphomycosis	*Cladophialophora bantiana*; species of *Alternaria*, *Cladosporium*, *Bipolaris*, *Exserohilum* and numerous other dematiaceous molds
		Mucormycosis (zygomycosis)	Species of *Rhizopus, Lichtheimia, Cunninghamella,* and other zygomycetes
		Pneumocystis pneumonia	*Pneumocystis jiroveci*
		Penicilliosis	*Penicillium marneffei*

SUPERFICIAL MYCOSES

1. *Malassezia Furfur* _ is dimorphic and part of normal skin flora. It is lipophilic yeasts, and most require lipid in the medium for growth.

Disease

Pityriasis Versicolor (aka Tinea Versicolor)

- Is a mild superficial infection
- chronic mild superficial infection of the stratum corneum
- Invasion of the cornified skin and the host responses are both minimal.
- Lesions occur mainly on back, chest, abdomen and neck.
- The lesions are either depigmented or brownish-red – especially seen on tanned skin during summer.
- The lesions are chronic and occur as macular patches of discolored skin that may enlarge and coalesce, but scaling, inflammation, and irritation are minimal.
- Indeed, this common affliction is largely a cosmetic problem.
- There might be slight scalding or itching, but usually infection is asymptomatic.
- They occur frequently in hot, humid weather and are only of cosmetic importance (may be ugly but not dangerous).
- Lesions contain both budding yeast cells and hyphae.

Lab diagnosis

- Direct microscopic examination of scrapings of infected skin, treated with 10-20% of KOH preparation or stained with calcofluor white. Short unbranched hyphae and spherical cells are observed.
- The lesions also fluoresce under Wood's lamp.
- Culture not usually done.

Treatment

- Topical miconazole or 1% selenium sulfide cream.
- Lesions have a tendency to recur so permanent cure is difficult to achieve.
- Rarely, *Malassezia* may cause an opportunistic fungemia in patients—usually infants—receiving total parenteral nutrition, as a result of contamination of the lipid emulsion.
- In most cases, the fungemia is transient and corrected by replacing the fluid and intravenous catheter.
- Some individuals develop folliculitis due to *Malassezia*.
- Species of *Malassezia* are considered part of the cutaneous microbiota and can be isolated from normal skin and scalp.
- They have been implicated as a cause of or contributor to seborrheic dermatitis, or dandruff.
- This hypothesis is supported by the observation that many cases are alleviated by treatment with ketoconazole.

2. Exopnilia Wemekii

Disease

Tinea Nigra

- Tinea nigra (or tinea nigra palmaris) is a superficial chronic and asymptomatic infection of the stratum corneum.
- This condition is more prevalent in warm coastal regions and among young women.

- Dark brown-black painless patches on soles of hands and feet.

Lab diagnosis

- Skin scrapings in KOH preparation.
- Microscopic examination of skin scrapings from the periphery of the lesion will reveal branched, septate hyphae and budding yeast cells with melanized cell walls.
- Culture not usually done.

Treatment

- Tinea nigra will respond to treatment with keratolytic solutions, salicylic acid, or azole antifungal drugs.

3. *Piedraia hortae*

Disease

Piedra

- Black piedra is a nodular infection of the hair shaft caused by *Piedraia hortae*.
- White piedra, due to infection with *Trichosporon* species, presents as larger, softer, yellowish nodules on the hairs.
- Axillary, pubic, beard, and scalp hair may be infected.
- Treatment for both types consists of removal of the infected hair and application of a topical antifungal agent.
- Piedra is endemic in tropical countries.

SUBCUTANEOUS MYCOSES

The fungi that cause subcutaneous mycoses normally reside in soil or on vegetation. They enter the skin or subcutaneous tissue by traumatic inoculation with contaminated material. For example, a superficial cut or abrasion may introduce an environmental mold with the ability to infect the exposed dermis. In general, the lesions become granulomatous and expand slowly from the area of implantation. Extension via the lymphatics draining the lesion is slow except in sporotrichosis. These mycoses are usually confined to the subcutaneous tissues, but in rare cases they become systemic and produce life-threatening disease.

1. Sporothrix Schenkii

- *Sporothrix schenckii* is a thermally dimorphic fungus that lives on vegetation.
- It is associated with a variety of plants—grasses, trees, sphagnum moss, rose bushes, and other horticultural plants.
- At ambient temperatures, it grows as a mold, producing branching, septate hyphae and conidia, and in tissue or in vitro at 35–37°C as small budding yeast.
- Following traumatic introduction into the skin, *S. schenckii* causes **sporotrichosis**, a chronic granulomatous infection.
- The initial episode is typically followed by secondary spread with involvement of the draining lymphatics and lymph nodes.

Morphology and Identification

- *Sporothrix schenckii* grows well on routine agar media, and at room temperature the young colonies are blackish and shiny, becoming wrinkled and fuzzy with age.
- Strains vary in pigmentation from shades of black and gray to whitish. The organism produces branching, septate hyphae and distinctive small (3–5 μm) conidia, delicately clustered at the ends of tapering conidiophores.

- Isolates may also form larger conidia directly from the hyphae.
- *Sporothrix schenckii* is thermally dimorphic, and at 35°C on a rich medium it converts to growth as small, often multiply budding yeast cells that are variable in shape but often fusiform (about 1–3 × 3–10 μm).

Epidemiology

- *Sporothrix schenckii* occurs worldwide in close association with plants.
- For example, cases have been linked to contact with sphagnum moss, rose thorns, decaying wood, pine straw, prairie grass, and other vegetation.
- About 75% of cases occur in males, either because of increased exposure or because of an X-linked difference in susceptibility.
- The incidence is higher among agricultural workers, and sporotrichosis is considered an occupational risk for forest rangers, horticulturists, and workers in similar occupations.

Antigenic Structure

- Heat-killed saline suspensions of cultures or carbohydrate fractions (**sporotrichin**) will elicit positive delayed skin tests in infected humans or animals.
- A variety of serologic tests have been developed, and most patients, as well as some normal individuals, have specific or cross-reactive antibodies.

Pathogenesis and Clinical Findings

- The conidia or hyphal fragments of *S schenckii* are introduced into the skin by trauma. Patients frequently recall a history of trauma associated with outdoor activities and plants.
- The initial lesion is usually located on the extremities but can be found anywhere (children often present with facial lesions).
- About 75% of cases are lymphocutaneous; that is, the initial lesion develops as a granulomatous nodule that may progress to form a necrotic or ulcerative lesion.
- Meanwhile, the draining lymphatics become thickened and cord-like.
- Multiple subcutaneous nodules and abscesses occur along the lymphatics. Fixed sporotrichosis is a single nonlymphangitic nodule that is limited and less progressive.
- The fixed lesion is more common in endemic areas such as Mexico, where there is a high level of exposure and immunity in the population. Immunity limits the local spread of the infection.
- There is usually little systemic illness associated with these lesions, but dissemination may occur, especially in debilitated patients. Rarely, primary pulmonary sporotrichosis results from inhalation of the conidia.
- This manifestation mimics chronic cavitary tuberculosis and tends to occur in patients with impaired cell-mediated immunity.

Disease

- **Sporotrichosis**
  - Fungi enters skin through lesions, typically through *thorn* stick _ causes local pustule or ulcer with nodules along the draining lymphatic.
  - Lesions may be chronic.
  - There are little systemic symptoms.

Lab diagnosis

- **Specimens**

Specimens include biopsy material or exudate from granulomatous or ulcerative lesions.

- **Microscopic Examination**

It can be examined directly with KOH or calcofluor white stain, the yeasts are rarely found.

Even though they are sparse in tissue, the sensitivity of histopathologic sections is enhanced with routine fungal cell wall stains, such as Gomori methenamine silver, which stains the cell walls black, or the periodic acid-Schiff stain, which imparts a red color to the cell walls.

Alternatively, they can be identified by fluorescent antibody staining. The yeasts are 3–5 μm in diameter and spherical to elongated.

Another structure termed an asteroid body is often seen in tissue, particularly in endemic areas such as Mexico, South Africa, and Japan.

In hematoxylin and eosin-stained tissue, the asteroid body consists of a central basophilic yeast cell surrounded by radiating extensions of eosinophilic material, which are depositions of antigen–antibody complexes and complement.

- Microscope investigation _ see characteristic cigar-shaped yeast.
- In culture _ hyphae with oval conidia in clusters at tip of conidiophores (looks like a daisy).

- **C. Culture**

The most reliable method of diagnosis is culture. Specimens are streaked on inhibitory mold agar or Sabouraud's agar containing antibacterial antibiotics and incubated at 25–30°C. The identification is confirmed by growth at 35°C and conversion to the yeast form.

- **D. Serology**

High titers of agglutinating antibodies to yeast cell suspensions or antigen-coated latex particles are often detected in sera of infected patients. However, these tests are generally not useful because elevated titers do not develop early in the course of disease and uninfected or previously exposed patients may give false-positive results.

Treatment

- In some cases, the infection is self-limited. Although the oral administration of saturated solution of potassium iodide in milk is quite effective, it is difficult for many patients to tolerate.
- The treatment of choice is oral itraconazole or another azole. For systemic disease, amphotericin B is given.

Prevention

- Protect skin when dealing with plants, moss or wood.
- Prevention includes measures to minimize accidental inoculation and the use of fungicides, where appropriate, to treat wood. Animals are also susceptible to sporotrichosis.

2. CHROMOBLASTOMYCOSIS

Chromoblastomycosis (chromomycosis) is a subcutaneous mycotic infection that is usually caused by traumatic inoculation of any of the recognized fungal agents, which

reside in soil and vegetation. All are dematiaceous fungi, having melanized cell walls: *Phialophora verrucosa, Fonsecaea pedrosoi, Fonsecaea compacta, Rhinocladiella aquaspersa,* and *Cladophialophora carrionii.* The infection is chronic and characterized by the slow development of progressive granulomatous lesions that in time induce hyperplasia of the epidermal tissue.

Phialophora verrucosa

- The conidia are produced from flask-shaped phialides with cup-shaped collarettes. Mature, spherical to oval conidia are extruded from the phialide and usually accumulate around it.
- Soil fungi
- Collectively called **Matia Ceous fungi** because they produce melanin like pigments.
- Enters skin via lesion.

Pathogenesis and Clinical Findings

Disease

- **Chromomycosis**
  - The fungi are introduced into the skin by trauma, often of the exposed legs or feet.
  - Over months to years, the primary lesion becomes verrucous and wart-like with extension along the draining lymphatics.
  - Cauliflower-like nodules with crusting abscesses eventually cover the area. Small ulcerations or "black dots" of hemopurulent material are present on the warty surface.
  - Rarely, elephantiasis may result from secondary infection, obstruction, and fibrosis of lymph channels.
  - Dissemination to other parts of the body is very rare, though satellite lesions can occur due either to local lymphatic spread or to autoinoculation.
  - Histologically, the lesions are granulomatous and the dark sclerotic bodies may be seen within leukocytes or giant cells.

Diagnostic Laboratory Tests

- Specimens of scrapings or biopsies from lesions are placed in 10% KOH and examined microscopically for dark, spherical cells.
- Detection of the sclerotic bodies is diagnostic of chromoblastomycosis regardless of the etiologic agent.
- Tissue sections reveal granulomas and extensive hyperplasia of the dermal tissue.
- Specimens should be cultured on inhibitory mold agar or Sabouraud's agar with antibiotics.
- The dematiaceous species is identified by its characteristic conidial structures, as described above.
- There are many similar saprophytic dematiaceous molds, but they differ from the pathogenic species in being unable to grow at 37°C and being able to digest gelatin.

Treatment

- Surgical excision with wide margins is the therapy of choice for small lesions.

- Chemotherapy with flucytosine or itraconazole may be efficacious for larger lesions. The application of local heat is also beneficial. Relapse is common.
- Infection treated with keratoylytic agents e.g. salicylic acid.
- Disease treated with oral flucytosine or thiabendazole.

Epidemiology

- Chromoblastomycosis occurs mainly in the tropics. The fungi are saprophytic in nature, probably occurring on vegetation and in soil.
- The disease occurs chiefly on the legs of barefoot agrarian workers following traumatic introduction of the fungus.
- Chromoblastomycosis is not communicable. Wearing shoes and protecting the legs probably would prevent infection.

3. <u>Mycetoma</u>

Mycetoma is a chronic subcutaneous infection induced by traumatic inoculation with any of several saprophytic species of fungi or actinomycetous bacteria that are normally found in soil.

The fungal agents of mycetoma include, among others, *Pseudallescheria boydii* (anamorph, *Scedosporium apiospermum*), *Madurella mycetomatis, Madurella grisea, Exophiala jeanselmei,* and *Acremonium falciforme.*

Madurella species

- Soil fungi.
- Dematiaceous molds
- Enters through wounds on hands, feet or back.
- These molds are identified primarily by their mode of conidiation.

Disease

- **Mycetoma**
  - In tissue, the mycetoma granules may range up to 2 mm in size.
  - The color of the granule may provide information about the agent.
  - Causes abscess with pus discharging through sinuses.
  - Puss contains colored granules.
  - *Madurella mycetomatis* produces a dark red to black granule.
  - These granules are hard and contain intertwined, septate hyphae (3–5 μm in width).
  - The hyphae are typically distorted and enlarged at the periphery of the granule.

Pathogenesis and Clinical Findings

- Mycetoma develops after traumatic inoculation with soil contaminated with one of the agents.
- Subcutaneous tissues of the feet, lower extremities, hands, and exposed areas are most often involved.
- Regardless of the agent, the pathology is characterized by suppuration and abscesses, granulomata, and draining sinuses containing the granules.
- This process may spread to contiguous muscle and bone. Untreated lesions persist for years and extend deeper and peripherally, causing deformation and loss of function.

Lab diagnosis

- Granules can be dissected out from the pus or biopsy material for examination and culture on appropriate media. The granule color, texture, and size and the presence of hyaline or pigmented hyphae (or bacteria) are helpful in determining the causative agent. Draining mycetomas are often superinfected with staphylococci and streptococci.

Treatment

- The management of eumycetoma is difficult, involving surgical debridement or excision and chemotherapy.
- Itraconazole, ketoconazole, and even amphotericin B can be recommended for *Madurella* infections.
- Chemotherapeutic agents must be given for long periods to adequately penetrate these lesions.
- No effective drug against fungal form.
- Surgical extension recommended.

Systemic mycoses.

- These infections result from ***inhalation of spores*** or dimorphic fungi, that have their saprophytic mold form in the ***soil*** (source), into ***respiratory tract (site of entry)*** Inside lungs, the spores differentiate into yeast or other specialized forms.
- Most lung infections are asymptomatic and self limiting, but in some people disseminated diseases develop where the organism grows in other organs and causes destructive lesions and may cause death.
- Infected people do not infect others.

Coccidioides immitis

Properties

- Dimorphic fungus – exists are mold in soil and **spherule in tissue** (it is also found in spherule form inside human body).
- In soil it forms arthrospores, which are very light and acre carried by the wind _can be inhaled and infect the lungs.
- Is endemic in southwestern USA and Latin America.

Disease

Coccidioidomycosis

- In the lungs, the arthrospores form large spherules with *thick, double refractive wall* and filled with *endospores*. When the wall ruptures, the endospores are released and form new spherules.
- The fungus can spread with direct extension or via blood flow.
- Granulomatous lesions can occur anywhere, but are mainly found in bones and CNS (meningitis).
- The infection of the lungs is usually *asymptomatic* and can only be proven by a *positive skin test* and presence of antibodies.
- Some people have influenza like symptoms (fever ad cough), ~50% have changes in the lungs that can be seen on X-ray and ~10% develop erythema nodosum or arthralgia _ this syndrome is called “Valley fever” or “Desert Rheumatism” and tends to subside spontaneously.
- Erythema nodosum manifests as red, tender nodules on extensor surfaces like shins _ it is a delayed hypersensitivity reactions to fungal antigens and thus is a good prognosis. It is not specific for coccidioidomycosis, it can also occur in other granulomatous diseases as well (tuberculosis, leprosy etc).

- Dissemination of disease indicates a defect in cell-mediated immunity. Most people who develop a positive skin test to infection will develop immunity against spread and reinfection, but if their cellular immunity is suppressed by drugs or other diseases, then dissemination can occur at any time. Pregnant women in third trimester have increased incident of dissemination.

Lab diagnosis

- Microscopically – will see spherules in skin specimen.
- Culture on Sabouraud agar – shows hyphae with arthrospores.
- Skin test with fungal extracts (coccidioidin or spherules)– becomes positive with him 2-4 weeks, but is often negative (anergy) in patients with disseminated disease.

Treatment

- *Amphotericin B*, for persisting lung lesions or disseminations.

Prevention

- No means, except avoiding travels to endemic areas.

Histoplasma capsulatum

Properties

- Is a *dimorphic fungus* – exists as *mold in soil* and *yeas in tissue*. It forms 2 types of asexual spores:

  1. **Tuberculate Macroconidia** – with thick walls and fingerlike projections.

  2. **Microconidia** – smaller, with thin, smooth walled spores that if inhaled will give infection.

- The fungus grows in soil, especially if soil is heavily contaminated with bird feces.
- Bats can also excrete the organism in their guano.

Disease

Histoplasmosis.

- The spores are inhaled _ engulfed by macrophages _ develop into yeast forms in tissue. In the tissue, H. Capsulatum occurs as oval budding yeast inside macrophages.
- The organism spreads widely throughout the body, especially to liver and spleen. But most infections remain asymptomatic, and the small granulomatous foci heal by calcification.
- With intense exposure (e.g. in chicken house or bat infested caves), pneumonia may become a clinical manifestation.
- Severe disseminated histoplasmosis develops in small minority of people, especially infants, elderly and immunocompromised people.

Lab diagnosis

- Microscopically – oval yeast can be seen inside macrophages in tissue biopsy or bone marrow aspirates.
- Culture on Sabouraud agar – shows tuberculate macroconidia.
- Skin test with fungal extract (histoplasmin) – becomes positive (induration) within 2-3 weeks of infection and remains positive for years. It may be negative in disseminated disease.
- Complement fixation and Immunodiffusion test – useful but cross react with other fungi, especially Blastomyces.
- Immunodiffusion test is more specific but less sensitive than complement fixation.

Treatment

- No therapy needed in asymptomatic or mild primary infection.

- ŏ Oral ketokonazole _ in progressive lung lesion.
- ŏ Amphotericin B _ in disseminated cases.
- ŏ Oral intraconazole _ in pulmonary or disseminated disease.

Blastomyces dermatidis

Properties

- Ψ A *dimorphic fungi* – exists as *mold in soil* and *yeast in tissue*.
- Ψ The *yeast* is round with a double refractive wall and a *single broad based bud* (C. Neoformans form narrow based bud).
- Ψ In endemic in North and Central America and in Africa.
- Ψ It grows mostly in moist soil rich in organic material (rotting vegetation etc) – here it
- Ψ forms hyphae with small pear shaped conidia.
- Ψ Infection occurs in *respiratory tract* through inhalation.

Disease

Blastomycosis.

- Ψ Infection is *asymptomatic* or mild (barely recognized).
- Ψ Dissemination may result in ulcerated granulomas of *skin*, *bone* or other sites.

Lab diagnosis

- Ψ Microscopically – thick walled yeast cells with single broad based bud can be seen in tissue biopsies.
- Ψ Culture – will show hyphae with smaller pear shaped conidia.
- Ψ Skin test – lacks specificity and has little value.
- Ψ Serologic test – have little value as well.

Treatment

- Ψ Local or oral *ketokonazole* in patient with lesions.
- Ψ Oral *intraconazole* can be used to treat pulmonary or disseminated disease.
- Ψ Surgical excision may be useful.

Paracoccidioides brasiliensis

Properties

- Ψ Is a dimorphic fungus – exists as mold in soil and as yeast in tissue.
- Ψ The yeast is thick walled with multiple buds, in contrast to B. Dermatidis, which has a single bud.
- Ψ It grows in soil.
- Ψ Is endemic in Latin America – diseases occurring only in this region.

Disease

Paracoccidioidomycosis

- Ψ Spores are inhaled and early lesions occur in the lungs.
- Ψ Asymptomatic infection is common
- Ψ Sometimes oral mucous membrane lesions, swollen lymph nodes and disseminations to other organs may develop.

Lab diagnosis

- Ψ *Microscopically* – yeast cells with multiple buds can be seen in puss or in tissues.
- Ψ Specimen *culture* – grown for 2-4 weeks may show typical organisms.
- Ψ Skin test – rarely helpful.
- Ψ *Serology* (*Immunodiffusion, complement fixation*) – shows that when significant antibody titers are found, active disease is present.

Treatment

Ψ Ketokonazole – taken orally for several months.

Opportunistic Mycoses

- Opportunistic fungi don't induce disease in most normal, immunocompetent people, but can cause severe infection in those with impaired host defenses.

Candida

- **Species:** *C. Albicans (most important), C. Tropicalis, C. Glabrata, C. Krusei*.

C. albicans

Properties

- Is an oval yeast with a single bud.
- It is part of *normal flora of mucous membrane* of *upper respiratory tract*, *G.I. tract* and *female genital tract.* It is not transmitted as part of normal flora – when local or systemic host defenses are impaired, disease may result.
- In tissue it may appear as budding yeasts or as elongated budding "*pseudohypae*".

Disease

1. Thrush _ overgrowth of C. Albicans in mouth produces *white patches*.

2. *Vulvovaginitis* _ associated with mucosal surfaces, with itching and discharge. It favored by high pH, diabetes or use of antibiotics.

3. Chronic mucocutanous candidiasis _ dissemination of candida to different organs often seen in immunocompromised patients (AIDS).

- Characteristic manifestations of candida infection in AIDS are: generalized oral candidiasis (GOC), oesophagitis, endocarditis and sepsis.
- Skin invasion occurs in warm, moist areas of body _ these parts before *red* and weeping.
- Fingers and nails become involved when repeatedly immersed in water (people employed as dishwashers re commonly affected) _ painful thickening/loss of nails.

Lab diagnosis

- Microscopically – see budding yeast or pseudohypae in exudates or tissue.
- Germ tubes form in serum at 37C _ this helps to distinguish C. Albicans from most other Candida species e.g. Chlamydospores are typically formed by C. Albicans, but not by the other.
- Skin test with candida antigens – uniformly positive in normal adults and are used as an indicator of competent cellular immunity.
- Serology – rarely helpful.

Treatment

- Local infection (e.g. thrush) – with antifungal drugs e.g. Clotrimazole and Nystatin.
- Disseminated candidiasis – Amphotericin B with or without Flucytosine or Ketokonazole.
- Treatment of candida infection with antifungal drugs should be supplemented by reduction of predisposing factors.

Prevention

- Certain candida infections e.g. thrush, can be prevented by oral Clotrimazole or Nystatin.

Cryptococcus neoformans

Properties

- Is an oval budding yeast surrounded by a **wide polysaccharide capsule** (it is the only one with capsule) – it is not dimorphic!
- The yeast occurs widely in nature and grows in large numbers n soil containing bird feces (especially pigeon droppings).
- Humans are infected by inhalation of the fungus.

Disease

Cryptococcosis

- Lung infection if is often asymptomatic or may produce pneumonia.
- Disease occurs mainly in immunocompromised people _ the organism disseminates to CNS and causes **meningitis** + infection of other organs.

Lab diagnosis

- Microscopically – sample of spinal fluid mixed with Indian ink shows yeast cells surrounded by a wide unstained capsule.
- Fungus can be cultured from spinal fluid + other specimens.
- Serology test can be done for both antibody and antigen.
- In infected spinal fluid capsular antigen occurs in high titer.

Treatment

- Combination of Amphotericin B and Flucytosine is used in meningitis or other disseminated disease.
- Fluconazole is used in AIDS patients for long-term suppression.

Aspergillus

Species: *A. Fumigatus, A. Flavus.*

Properties

- Aspergillus species occur only as mold, they are not dimorphic.
- They have septatet hyphae that form V-shaped branches. The walls are more or less parallel (in contrast to Mucor or Rhizopus wall which are irregular).
- The conidia of aspergillus form radiating chains.
- The molds are widely distributed in nature – they grow in decaying vegetation chains of conidia.
- Transmission is by airborne conidia.

Disease

Aspergilliosis

- Fumigatus can colonize and later invade abraded skin, wounds, burns, the cornea, external ear or paranasal sinuses.
- In immunocompromised person, it can evade the lungs + other organs and produce hemoptysis and granulomas.
- Aspergillus can grow in pulmonary cavities and produce a fungus ball, which can be seen on X-rays. Can also cause allergic asthma.
- Flavus, which grows on cereals or nuts produces afla toxins _ may be carcogenic or acutely toxic.

Different stages of Pulmonary Aspergilliosis:

1. Aspergillus ball (in performed cavities).

2. Invasive aspergilliosis (in immunosupression).

3. Allergic bronchopulmonary aspergilliosis.

Lab diagnosis

- ♣ Microscopically – the biopsy specimen shows septate, branching hyphae.
- ♣ Culture – shows colonies with characteristic radiating chains of conidia. But a positive culture does not prove disease, because colonization is common.
- ♣ Serology – shows that in people with allergic asthma there is high levels of IgE.
- ♣ In people with invasive aspergilliosis, there may be high titer of galatcomannan antigen in serum.

Treatment

- ♣ Amphotericin B – for invasive aspergilliosis.
- ♣ Surgical removal of fungus ball growing in a sinus or in a pulmonary cavity.
- ♣ No specific means of prevention.

UNIT-4
Viral Infections

Origin:

- The origin of modern viruses is not entirely clear. It may be that no single mechanism can account for their origin. Small viruses with only a few genes may be runaway stretches of nucleic acid originating from the genome of a living organism. Their genetic material could have been derived from transferable genetic elements such as *plasmids* or *transposons*, that are prone to moving within, leaving, and entering genomes. New viruses are emerging *de novo* and therefore, it is not always the case that viruses have "ancestors".
- Viruses with larger genomes, such as *poxviruses*, may have once been small cells that parasitized larger host cells. Over time, genes not required by their parasitic lifestyle would have been lost in a streamlining process known as *"retrograde-evolution" or "reverse-evolution"*.
- The bacteria *Rickettsia* and *Chlamydia* are living cells that, like viruses, can only reproduce inside host cells. They lend credence to the streamlining hypothesis, as their parasitic lifestyle is likely to have caused the loss of genes that enabled them to survive outside a host cell. It is possible that viruses represent a primitive form of self replicating DNA and are a precursor to life as it is currently defined. Other infectious particles which are even simpler in structure than viruses include viroids, satellites, and prions.

Structure and Chemical composition:

Viruses are:

- *Non cellular*
- Have no *nucleus, ribosomes or mitochondria* _ so they depend on the host for protein synthesis and ATP production
- Have *no motility*
- Replicate by *binary fission* (= asexual reproduction which involves the splitting of the parent cell into two progenies).
- Virus means “poison”

Size:

- 20 – 300 nm in diameter. (Smallest virus is Parvovirus, largest is Filovirus/Poxvirus)

Nucleic acid:

- **RNA viruses;** 3 types:
  - ***Positive stranded*:** - acts like mRNA _ can be translated directly by host’s ribosome into proteins. Can be single or double stranded
  - ***Negative stranded*:** - has to first be transcribed into a positive strand by RNA dependent RNA polymerase (found in virus’ capsid), then translated into proteins, etc. Can be single or double stranded.
  - ***RNA of retroviruses*:** - the RNA is transcribed in a reverse way; first transcribed into DNA by reverse transcriptase, then transcribed into mRNA, then finally translated into structural proteins and enzymes.
    Humans do not have RNA dependent RNA polymerase!
- **DNA viruses;**
  - Have to first be transcribed into mRNA by *DNA dependent RNA polymerase* before it can be translated into structural proteins and enzymes.

- They have both positive and negative strands. The positive strand is used to make mRNA while the negative strand is ignored.
- DNA is always a single molecule, while RNA can be segmented. So nucleic acid can be:

- Single or double stranded
- DNA or RNA (never both!)
- Linear or circular
- DNA is always a single molecule
- RNA is always segmented
- Almost all viruses are haploid (Retrovirus is diploid)

Capsid and symmetry:

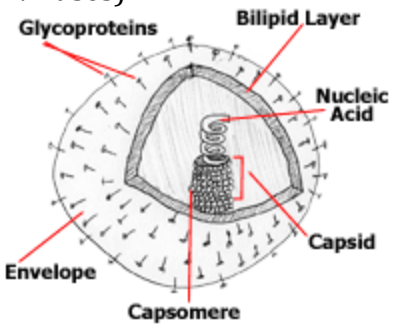
- Capsid is a protein coat that surrounds the nucleic acid.
- Their subunits are capsomeres which consist of one or more proteins.
- It is the way the capsomeres are arranged which gives the viruses their distinct shape:

1. Icosahedral:

- Triangle shaped
- Either DNA or RNA viruses
- Either enveloped or naked

2. Helical:

- Hollow, coil arrangement
- Only RNA viruses have this arrangement
- All enveloped (no naked helical viruses)



Importance of capsid:

- Protects the genetic material;
- Mediates attachment of virus to specific receptors on host cells.
- The virus proteins and the host cell receptors are the major determinants of species and organ specificity.
- The outer viral proteins also act as important Ags _ they induce neutralizations of antibodies and activate cytotoxic Tcells to kill virus infected cells.
- Host antibodies can bind to the viral proteins and stop the virus from entering the cell and replicating.

Envelope:

- It is a lipoprotein membrane that is derived as the virus exits the cell during "budding".
- Its proteins are virus specific and important for serotype (type specific) in order to know what vaccine to use (e.g.Poliovirus 1, 2, 3).
- Its glycoproteins will attach to the host cell receptors during entry of virus into cell.
- Its matrix proteins form an interaction between capsid and envelope
- Viruses with envelopes are less stable, meaning they are more easily inactivated + more sensitive to heat detergents and lipid solvents than the naked viruses.
- Thus the enveloped viruses are transmitted by direct contact (blood, body fluids), while the naked viruses can survive longer in the environment and can be transmitted indirectly (fecal-oral).
- Non enveloped viruses consist only of nucleic acid and capsid proteins.

Classification of viruses

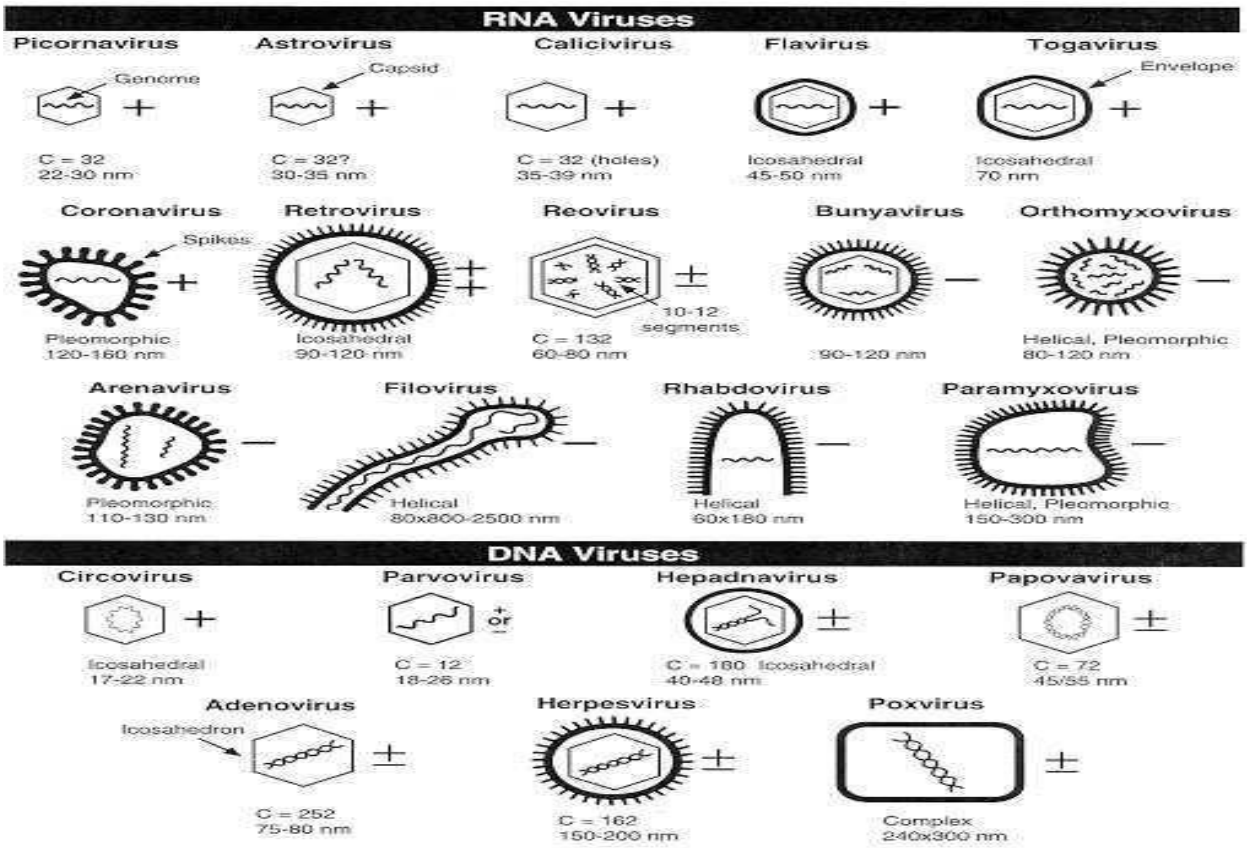
Viruses are classified according to:

- Nucleic acid:
  - type: DNA or RNA
  - double stranded/single stranded
  - single or segmented pieces of nucleic acid
  - positive or negative stranded RNA
  - complexity of genome
- Capsid:
  - icosahedral
  - helical
  - Envelope:
  - naked
  - enveloped
- Size:
  - diameter of helical capsid virus
  - number of capsomeres in icosahedral capsid.

Atypical viruses:

- **Defective viruses**: Need a helper virus to replicate. They have a mutation or deletion as part of their nucleic acid. E.g. Hepatitis D.
- **Pseudoviruses:** They contain host DNA. They can infect cells, but can't replicate.
- **Viroids:** single molecule of circular RNA without protein coat or envelope. It can replicate but doesn't code for only proteins. Important in plant diseases.

Prions: are infectious particles that consist of only proteins (have no DNA or RNA!). They are more resistant to heat and UV light than regular viruses, have no immune or inflammatory response and are important in slow diseases "transmissible spongiform encephalopaties" e.g. Creutzfeldt-Jacob disease.



Influenza virus.

Characteristics

- Belongs to Orthomyxoviridae
- Enveloped RNA virus, single stranded, segmented RNA with negative polarity.
- Has RNA dependent RNA polymerase _ transcribes negative genome into mRNA (the genome is therefore not infectious)
- Has 3 subtypes: *A, B and C*

Its two main antigens are

- Hemagglutinin (HA) _ binds virus to cell surface receptor + agglutinates RBC.
- Neuroaminidase (NA) _ degrades the mucous layer in resp. tract.
- *Antigenic shift in these proteins due to change of RNA segments results in the epidemics caused by influenza A, only!*
- Influenza A virus of animals are the source of the new RNA segment
- There are **also *antigenic drifts (=mutations) seen in A and B***. Due to these antigenic shifts and drifts, the virus has *allot of serotypes* _ this is why influenza virus keep recurring time and time again.

Influenza antigens are divided into:

- **Group specific**: the internal nucleocapsid protein _ will determine whether the virus is A, B or C.
- **Type specific**: hemaglutinin, neuraminidase.

Replication

- Binds via HA to cell surface receptor _ enters cell in a vesicle _ uncoats _ transcribes mRNA _ translates mRNA into proteins in cytoplasm _ progeny RNA genome are synthesized in *nucleus* _ assembly of virions in *cytoplasm* _ virions released by *cell budding*.
- Influenza and retrovirus are the only RNA viruses that have an important stage of replication taking place in nucleus!

Pathogenesis

- Progeny virions are soon produced and spread to adjacent cells, where the replicative cycle is repeated. Viral NA lowers the viscosity of the mucous film in the respiratory tract, laying bare the cellular surface receptors and promoting the spread of virus-containing fluid to lower portions of the tract. Within a short time, many cells in the respiratory tract are infected and eventually killed.
- The incubation period 1 day to 4 days.
- Preceding onset of symptoms peaks within 24 hours, remains elevated for 1–2 days, and then declines over the next 5 days.
- Infectious virus is very rarely recovered from blood.
- Interferon is detectable in respiratory secretions about 1 day after viral shedding begins. Influenza viruses are sensitive to the antiviral effects of interferon, Specific antibody and cell-mediated responses cannot be detected for another 1–2 weeks.
- Influenza infections cause cellular destruction and desquamation of superficial mucosa of the respiratory tract but do not affect the basal layer of epithelium. Complete reparation of cellular damage probably takes up to 1 month.
- Edema and mononuclear infiltrations in response to cell death and desquamation caused by viral replication probably account for local symptoms.

The prominent systemic symptoms associated with influenza probably reflect the production of cytokines.

Disease

- **Influenza A**: - ***H1N1 and H3N2 are the A subtypes that mainly cause human infections.***
- Cause worldwide epidemics (pandemic) of infections.
- **Influenza B**: causes major outbreaks, not pandemics.
- **Influenza C**: mild resp. tract infections.

Transmission

By airborne respiratory droplets

Pathogenesis

- Affects mainly the epithelium of resp. tract.
- Systemic symptoms are due to circulating cytokines.
- Immunity is provided by secretory IgA (IgG less protective), Cytotoxic T-cells and antibodies against neuraminidase.

Clinical findings

- Incubation period ~ 24-28 hours
- Fever, headache, myalgia (muscle pain) and cough _ symptoms usually resolve within 4-7days
- Aspirin will decrease fever, but can result in Reyes syndrome in children

Lab diagnosis

- Virus which is grown in cell culture or embryonated eggs can be detected by hemadsorption or hemagglutination
- Identification also by hemagglutination inhibition or complement fixation test
- 4 fold or more increase in antibody titer (serological)

Treatment

- ***Amantadine _ only effective for Influenza A (prevents penetration and uncoating)***
- Rimantadine & Neuraminidase inhibitors

Prevention

- **Killed virus vaccine – it contains 2 influenza A strains (H1N1, H3, N2) and 1 influenza B strain** _ it will induce IgG.
- Lasts only 6 months, Should be given to people above 65 and those with chronic diseases.

Measles virus

Characteristics

- Enveloped, helical, single stranded RNA virus with negative polarity, RNA polymerase
- Measles virus is spherical, but is often pleomorphic, measuring 120–250 nm in diameter.
- Single serotype
- Humans are natural hosts

Replication

- Penetrates and uncoats _ RNA polymerase transcribes negative strand into multiple mRNA _ mRNA translated into proteins _ assembly of nucleocapsids _ released by budding.

Transmission

- Respiratory droplets via upper resp. tract

Pathogenesis

- Infects upper resp. tract _ spreads via lymph nodes and then via blood _ infects reticuloendthelial cells where it replicates again _ enters blood again _ ends up in skin.
- A rash will results after infection of the skin, the rash is caused mainly by the cytotoxic T-cells attacking infected cells of the skin.
- After appearance of rash, the virus can no longer spread to other people.
- Multinucleated giant cells will form as a result of fusion (F) proteins in the spikes.
- Cell mediated immunity is important.
- Maternal Ab cross the placenta

Clinical findings

- Incubation period: 10-14 days
- Fever, conjunctivitis, runny nose, cough
- Koplik's spot _ red lesion with central white spot in buccal mucosa (diagnostic)
- Maculopappular rash on face _ migrates down the body, palms and soles of feet

Diseases

- Measles
- **Sub-acute sclerosing panencephalatis (SSP)** _ affects CNS and is a possible late complication of measles (years after primary infection)
- Primary and secondary measles pneumonia
- In pregnant women measles infection leads to still-birth

Lab diagnosis

- Based on clinical grounds (Koplik's spot + rash)
- Usually not isolated
- Serological testing useful
- 4 fold increase in antibody titer

Treatment

- Ribavirin given either intravenous or in aerosol form is being now evaluated to treat severely affected adults and immunocompromised individuals with acute measles.
- No antiviral therapy

Prevention

- Live attenuated vaccine usually combined with mump and rubella vaccine.
- Given subcutaneously to children of 15 months. Must not be given before this age as the maternal Abs are capable of neutralizing the virus before vaccine.
- Booster dose is recommended – vaccine wares off.

Dengue.

Characteristics and Replication

- Enveloped, single stranded, icosahedral nucleocapsid, positive polarity.
- The life cycle of the Arbovirus depends on the ability of these viruses to multiply in both vertebrae host and blood sucking vector.
- For the transmission to be effective, the virus has to be present in high amounts in the host's blood so that it can be taken up in small amounts by the insects.
- After the virus has been taken up by the vector (on the female insect serves as vector), it will replicate in the vector's gut and then spread to other organs, like the salivary glands.

- The extrinsic incubation period has to pass _ this is the time it takes for the virus to have replicated enough for the saliva of the vector to contain enough virus for it to be transmitted and be infectious. This takes ~ 7-14 days.
- Humans are usually dead end hosts, meaning a vector can't pass on the virus from one person to another. This is because the concentration of virus in the human blood is too low and the duration of viremia is too short for the next bite to transmit the virus.
- However, in some diseases, like dengue or yellow fever, humans have high doses of viremia so they act as reservoirs for the virus!
- Virus doesn't usually cause diseases in arthropod vector or vertebrae animal that serves as natural hosts (e.g. monkeys) – but do cause disease in dead-end-hosts (humans).

Transmission

- Via mosquitoes (Aedes mosquito vector).
- Reservoirs are humans (but jungle cycle involves monkeys as reservoirs).

Pathogenesis

- The leakage of plasma caused by increased capillary permeability is the major pathophysiological abnormality that occurs in dengue hemorrhagic fever and dengue shock syndrome. Bleeding, which is most important manifestation in patients with dengue hemorrhagic fever, is caused due to capillary fragility and thrombocytopenia, and it manifests by various ways ranging from petechial skin hemorrhages to life-threatening gastrointestinal bleeding.

Diseases

1) Classical Dengue = Breakbone Fever

- Occur in tropical areas all over the world.
- Starts with influenza like symptoms: fever, malaise, cough and headache _ progress to severe joint and muscle pain ("breakbone"). Enlarged lymphnodes, maculopapular rash and leukopenia are also common. Symptoms will regress after ~ 1 week.
- Has few sequela (pathological condition resulting from a disease) and is rarely fatal.

2) Dengue Hemorrhagic Fever

- Occurs in South Africa
- Much more severe. The initial picture is the same as the classic dengue, but then shock and hemorrhage in GI tract and skin develops.
- 10% fatality rate.

3) Hemorrhagic Shock Syndrome

- Occurs due to a second dengue infection and cross reaction of antibodies.
- Pathogenesis: the patient recovers from classical dengue (which has 4 serotypes) – antibodies for that serotype are then produced. So then when the patient gets infected with another serotype of dengue virus, it will result in anamnestic heterotypic response. In additions allot of cross reacting antibodies to the first serotype are produced.

After this there are 2 hypothesis of what happens next:

1. Immunocomplexes of virus and antibodies are formed that activate complement leading to increased vascular permeability and thrombocytopenia.
2. Antibodies will increase entry of viruses into monocytes and macrophages which will release large amounts of cytokines.

1. In both cases, shock and hemorrhage will result.

Lab diagnosis

- Blood collected during first 3–5 days of illness is useful for isolation of virus, and serum is useful for serological tests.
- Isolating virus in cell culture during first 3–5 days of illness.
- Serology; demonstrating presence of IgM antibodies capture ELISA (MacELISA) is the most widely used test.
- 4 fold increase in antibody titer in acute and convalescent phase.
- Neutralization test, hemagglutination inhibition, and IgG ELISA are the other serological tests used for diagnosis of the condition.

Treatment and Prevention

- No antiviral therapy or vaccine

Prevention and control

- No vaccine is available for prevention of dengue infection. The preventive measures are based mostly on mosquito- control activities. These include the use of insecticides and clearing the stagnant water and artificial collections of water that serve as breeding ground for the mosquitoes. Personal control measures include wearing good protective clothings and use of mosquito nets, mosquito repellants, etc.

Rabies virus

Characteristics

- Belongs to the Rhabdovirus family
- Enveloped, bullet shaped (helical), single stranded RNA with negative polarity, RNA-polymerase in virion
- Single serotype; Its antigenicity resides in the glycoprotein spikes
- Has wide host range

Replication

- Attaches to acetylcholine receptor on the cell surface _ enters cell and synthesizes 5 mRNAs that will code for *viral* proteins _ RNA polymerase will replicate the RNA (genome) _ the RNA that is produced will be assembled with virion proteins to form nucleocapsids _ virus leave the cell through budding (enveloped).

Diseases

Rabies _ it is an encephalitis (= acute inflammation of the brain)

Transmission

- ***Through bite of a rabid animal*** (rarely through aerosol)
- Rodents do not transmit rabies
- ***It will spread in the host along the axons***

Pathogenesis

- The virus will replicate at the site for the bite and infect *sensory neurons* _ it will spread by moving along the *axons to CNS* _ replicate in the *brain* _ then move down along the *peripheral nerves* to *salivary glands* (+ other organs) and enter the saliva _ from saliva it can be transmitted through bite (e.g. loose aggressive dogs).
- The CNS encephalitis will lead to death and demyelination of neurons.

Clinical findings

- Incubation time varies according to the bite site; how close it is to the CNS/brain correlates to how far the virus has to travel. So anything between 2.16 weeks, the closer the bite is to the CNS/brain the faster you will see symptoms.

- Non specific symptoms: fever + loss of sensation at bite site, develops into _ confusion, lethargy, increased saliva production _ painful spasm in throat muscles when swallowing _ seizure, paralysis, coma _ death (if not treated in time).

Lab diagnosis

- Detection of *Negri bodies* (cytoplasmic inclusions) in tissue samples (brain, skin, and hair) with fluorescent antibodies or with different dyes.
- Cell culture can be performed, but is too slow process
- Serological testing, but only useful in clinically ill patients
- Antibodies do not for quickly enough to help with diagnosis

Treatment

- No specific antirabies agent is available. Although until recently rabies was considered to be invariably fatal, it has now been demonstrated that complete recovery can occur from established rabies with intensive supportive care and management of complications.

Prevention

- Pre-exposure: immunization with killed virus vaccine (for cats and dogs etc)
- Post-exposure:
    - washing wound
    - ***give rabies immunoglobulin*** into the wound (**passive immunization)**
    - ***give human diploid cell vaccine* (*killed vaccine*)** at another site **(*active immunization)***
    - ***So to humans you give: - active immunization with human diploid cell vaccine (killed vaccine) and - passive immunization with rabies immunoglobulin.***

Hepatitis Viridae

- Viral hepatitis is an infection of the liver hepatocytes caused by viruses.
- The viruses are:
    - **Hep. A (HAV)**
    - **Hep. C (HCV)**
    - **Hep. D (HDV)** All RNA Viruses
    - **Hep. E (HEV)**
    - **Hep. G**
    - **Hep. B (HBV)** _ the only DNA virus!!
- Hep. A and E are transmitted through faecal-oral route, all the rest are transmitted via blood-to-blood (parenteral).

Acute Viral Hepatitis:

- This is a sudden illness with a mild to severe course followed by complete resolution.
- It can be caused by all the hep. viruses.
- Its incubation time varies – depends on the virus.
- As the virus grows it will give systemic symptoms that resemble the flue (fatigue, fever, runny nose, cough, aches etc).
- After 1-2 weeks the patient will develop jaundice and bilirubin levels will increase (bilirubin is usually cleared by the liver).

- As the virus continues to grow, the hepatocytes will die and will release the enzymes which are normally produced. These are liver-function-enzymes: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Gamma-glutamyl transpeptidase (GGT) and Alkaline Phosphatase. An increase in the level of these in the blood will help to diagnose hepatitis.
- So 2 weeks into the illness, the patient usually has jaundice, painful enlarged liver and high levels of liver function enzymes.

Chronic Liver Hepatitis

- It is a prolonged course of active disease or silent asymptomatic infections.
- Caused by the parenteral (blood-to-blood) viruses: HBV, HCV and HDV.
- Harder to diagnose because patient is usually asymptomatic with only an enlarged liver and slight increase in liver-function-enzymes.

HEPATITIS A VIRUS (HAV)

Characteristic

- Is a typical Enterovirus, classified in the Picornavirus family.
- Naked, single stranded RNA with positive polarity, thus no RNA polymerase in virion.
- One serotype.

Disease

- Hepatitis A _ common cold symptoms

Transmission

- Faecal-oral route
- Humans are reservoirs
- Children are mainly affected (at camp, boarding school etc)

Pathogenesis

- Virus replicate in GI tract _ enters blood _ liver. The mechanism of how the hepatocytes get damaged is unclear, but it is most likely due to the attack of cytotoxic T cells.
- Immune response consists mainly of IgM.

Clinical findings

- Same as HBV

Lab diagnosis

- You can find virus in stool 2 weeks prior to appearance of symptoms.
- In early phase of infection _ detection of virus by immune-EM from stool.
- Detection of IgM with RIA, ELISA, Vidas.
- From liver biopsy _ detection of virus antigen with immune-cytochemistry.

Treatment

- None

Prevention

- Vaccine containing killed virus (active immunization).
- Giving immune globulin (passive immunity) during incubation period can mitigate the disease.

HEPATITIS B VIRUS

Characteristics

- Member of Hepadnavirus family.

- Enveloped, partially circular (icosahedral), double stranded DNA virus, has DNA-polymerase in virion (see replication of DNA for more info!).
- It has 3 important antigens:
- Surface antigen – ***HBsAg***
- The core antigen – ***HBcAg***
- The e-antigen – ***HBeAg***
- HBcAg and HBeAg are both found in nucleocapsid, but are antigenically different.
- HBeAg is important indicator of transmissibility. Since it is found in the core protein, if detected, it shows that the virus must be in the patient's blood.
- Humans are the only natural hosts for HBV.

Transmission

- Sexual contact
- Perinatally from mother to newborn
- By blood or blood products

Pathogenesis

- Virus enters blood _ infects hepatocytes _ viral antigens get displayed on the surface _ cytotoxic T-cells mediate immune response against antigen _ inflammation and necrosis occurs.
- Since Hep. B does not cause cytopathic effect (CPE) itself, the pathogenesis of Hep. B is most likely due to the immune response of cytotoxic T-cells.
- The antigen-antibody complexes cause:
- arthritis
- rash
- glomerulonephritis
- Can cause chronic infection in: ***90% of infants*** **(most likely because immune system is less competent),** ***30% of children*** **and** ***6% of adults*****.**
- A chronic carrier is someone who has ***HBsAg*** persisting in their blood for at least 6 months.
- A high rate of ***hepatocellular carcinoma*** occurs in chronic state.
- Lifelong immunity occurs after natural infections – by humeral antibody against HBsAg HBsAb _ it will bind to surface receptors on virion and stops it from interacting with receptors on hepatocytes.

Disease

- Hepatitis B _ leads to hepatocellular carcinoma (in chronic carried state).

Clinical findings

- Many HBV infections are asymptomatic and can only be detected by presence of antibody to HBsAg.
- Incubation period ~ 10-12 weeks (longer than Hep. A).
- HBV symptoms are much more severe than HAV.
- HBV can lead to cirrhosis and death.

Lab diagnosis

- Electron microscope
- PCR (viral DNA in genome)
- In situ Ag detection (liver biopsy)
- Serology: ELISA, IFA.

- Prognostic evaluation of serological markers:
- ***HBsAg*** _ acute/chronic active disease.
- **Anti-HBsAg** _ ***shows protection (is never present in chronic state).***
- **HBeAg** _ gives bad prognosis due to high transmissibility.
- **Anti-HBeAg** _ covalence, lower transmissibility.
- **Anti-HBcAg** _ acute disease.
- ***So HBsAg, Anti-HBcAg and IgM are serological markers that prove acute disease.***

Treatment

- Alpha-interferon shown to help in chronic Hep. B. Other than that, no treatment.

Prevention

- Active immunization: recombinant HBsAg vaccine (subunit vaccine).
- Passive immunization: HBIG (Hep. B immune globulin).

HEPATITIS C

Characteristics

- Is a member of the Flavivirus family.
- Enveloped, single stranded RNA with positive polarity. No RNA polymerase in virion.
- Have about 6 genotypes and multiple sub-genotypes.

Replication

- It is uncertain how the virus replicates since it hasn't been grown in cell culture.
- Other Flavivirus replicates in the cytoplasm and translates RNA into large glycoproteins which are cleaved by protease –HBC might replicate the same way.
- Disease
- Hepatitis C _ associated with hepatocellular carcinoma.

Transmission

- Blood, sexually, parenterally (mother to child).

Pathogenesis

- Hepatocellular injury/death is probably caused by cytotoxic T cell immune response (because HCV doesn't cause CPE).
- Chronic state (50%) may occur leading to hepatocellular carcinoma.

Clinical findings

- Same as HBV.

Lab diagnosis

- Detected antibodies to HCV in ELISA
- RT-PCR: viral RNA in serum (active disease).
- Detection of increased transmission level.

Treatment

- Alpha interferons mitigate chronic hepatitis, but don't remove carrier state.

Prevention

- It is important to screen donated blood before giving transfusions.
- No vaccine or hyperimmune globulins available.

HEPATITIS D (DELTA)

Characteristics

- Enveloped, single stranded RNA virus with negative polarity, no virion polymerase because genome RNA is replicated and transcribed by host cell RNA polymerase.

- It is a detective virus, meaning it can't replicate by itself because it doesn't have the genes for its envelope proteins. It can only replicate in cells that are also infected with HBV _ because HDV will use the surface antigens of HBV (HBsAg) as its own envelope proteins. So HBV "helps" HDV.
- It has one serotype.

Disease

- Hepatitis D _ hepatocellular carcinoma.

Transmission

- Blood, sexually, parenterally (mother to child).

Pathogenesis

- Hepatocellular injury most probably cause by cytotoxic T cells, like in Hep. B.
- Chronic carrier state occurs _ hepatocellular carcinoma.

Clinical findings

- It can only occur in people that are already infected with HBV or in people previously infected with HBV and then superinfected with HDV.

Lab diagnosis

- Serology: anti-HDAg Igm antibodies
- Detecting HDAg and HDV RNS in patient's serum.

Treatment

- Alpha interferon mitigates symptoms but do not eradicate carrier state.

Prevention

- Preventions used for HBV (see previous notes) will also prevent HDV.

HEPATITIS E VIRUS (HEV)

- Naked, single stranded RNA virus.
- Is it classified as a member of the Caliciviruses.
- It is the main cause of enterically transmitted hepatitis.
- Is the common cause of water-borne epidemics of hepatitis in Asia, Africa, India and Mexico.
- Clinically resembles Hep. A, but chronic state does not occur.

Diagnosis:

- Serology: detection of IgM and IgG antibodies.
- In acute phase, virus RNA can be detected from feces.
- No antiviral treatment, no vaccine.

HIV.

Characteristics

- Enveloped virus with two copies (diploid) of single stranded RNA genome with positive polarity.
- The RNA dependent DNA polymerase (reverse transcriptase) will make a DNA copy of the genome, which it will then integrate into the hosts DNA.
- The precursor polypeptides get cleaved by virus encoded protease to produce functional viral proteins.
- Structural genes: *gag, pol, env* _ encode structural proteins.
- Regulatory genes: *tat, rev* _ needed for replication.
- gag encodes for protein p24 _ antigen used in serological tests!

Disease

- Acquired immunodeficiency syndrome (AIDS).

Transmission

- Via body fluids (blood, semen, vaginal).
- Transplacentaly and perinatal transmission.

Pathogenesis

- There are 2 receptors needed for HIV to enter the cell:
- **CD4 protein**
  - Found mainly on helper T-cells _ so HIV enters these cells, infects and kills them.
  - Killing of CD4 T-cells predisposes to opportunistic infections (astrocytes also carry CD4 cells and thus also get infected)
- **Chemokine receptors** like CCR5.
  - The *NEF protein* is an important virulence factor as it decreases MHC-1 protein synthesis and in this way reduces the ability of cytotoxic T-cells to kill HIV infected cells.
  - Cytotoxic T-cells are the main host defense against HIV!

Clinical findings

1. Acute stage:

- Starts 2-4 weeks after infection
- Fever, lethargy, soar throat, maculopapular rash on the trunk.
- Antibodies arise ~ 3-4 weeks after infection.
- Can determine "viral load" at this point.

2. Middle stage/Latent stage:

- Latent period lasts for years.
- Patient is asymptomatic
- Viremia is low or absent
- AIDS related complex syndrome (ARC) can occur during this period _ involves persistent fever, fatigue, weight loss and lymphadenopathy.
- ARC often progress into AIDS

3. Immunodeficient stage:

- manifested by decrease in CD4 to below 400/mm3
- prone to infections by:
- ***herpes simplex, cytomegalovirus etc***
- ***fungal infections (cryptococcal meningitis)***
- ***protozoal infection (toxoplasmosis)***
- ***bacterial infection (mycobacterium)***
- Common to develop: pneumocytosis pneumonia, Kaposi's sarcoma and neurological problems (dementia and neuropathy).

Lab diagnosis

- **Usually made by detecting antibodies, with ELISA as the screening test and Western blot as confirmatory test.**
- Can determine the about of HIV RNA in plasma ("viral load") by using PCR based assay. A high viral load means that you are more prone to a rapid progression to AIDS compared to a low viral load.
- p24 used as a serological marker.

Treatment

- **Nucleoside analogues** inhibit HIV replication by inhibiting reverse transcriptase:

- *Zidovudine (AZT)*
- *Dideoxyinozine (Didanosine)*
- *Dideoxycytidine (Zalcitabine)*
- *Stavudine*
- *Lamivudine*
- *Abacavir*

- **Non nucleoside inhibitors** of reverse transcriptase are also used:
  - *Nevirapine*
  - *Delavirdine*
  - *Efavirens*
- **Protease inhibitors** which inhibit cleavage of precursor polypeptides:
  - *Indinavir*
  - *Saquinavir*
  - *Ritonavir*
  - *Nelfinavir*
  - *Amprenavir*
- **"Highly active antiretroviral therapy" (HAART)** is also currently used in treatment of AIDS. It involves combination of two nucleoside inhibitors (like *Zidovudine* and *Lamivudine*) and a protease inhibitor (e.g. *Indinavir*).

Prevention

- Screening of blood for presence of antibodies prior to transfusion.
- Safe sex (condoms)
- AZT should be given to HIV infected mothers and their babies.
- No vaccine!

HERPES VIRUS FAMILY

1) Herpes simplex 1 and 2
2) Varicella Zoster virus (VZV)
3) Cytomegalovirus virus
4) Epstein Barr virus
5) HHV-6 and 8 (Human herpes virus 6 and 8)

Characteristics

- DNA enveloped
- Double or single stranded
- Icosahedral core
- Large diameter (120-200 nm)
- Replicates in nucleus
- Are the only virus that get their envelope by budding through nuclear membrane
- Ability to cause latent infection
- Primary infection is usually more severe than reactivation
- They are divided into 3 subgroups base on where they cause latent infection and the type of cells they infect:
  1. **Alpha herpesvirus**
     - Infect epithelial cells
     - Latency in neurons
     - HSV-1 and 2 and VZV
  2. **Beta herpesvirus**

- ♣ Latent in different tissues
- ♣ Cytomegalovirus and HHV-6

3. **Gamma herpesvirus**
   - ♣ Infection and latency mainly in lymphoid cells
   - ♣ Epstein Barr virus and HHV-8

HERPES SIMPLEX VIRUS 1 &2

Characteristics

- ♣ HSV1 and HSV2 are the same structurally and morphologically, they are distinguished based on antigenicity and location of lesion.

Diseases

- ♣ HSV1
- ♣ Oropharyngeal herpes (gingivostomatitis, herpes labialis = cold sores)
- ♣ Keratoconjuctivitis
- ♣ Encephalitis
- ♣ Herpes genitalis
- ♣ HSV2
- ♣ Genital herpes
- ♣ Aseptic meningitis
- ♣ Neonatal complications:
- ♣ Disseminated infection
- ♣ Encephalitis
- ♣ Skin, eye and eye infection

Transmission

HSV1:

- ♣ Saliva or direct contact with virus from vesicle

HSV2:

- ♣ Sexual contact and through birth canal in neonates
- ♣ Transmission usually happens when lesions are present. Infection by HSV1 mainly appears during childhood while HSV2 infections don't appear until age of sexual activity.

Pathogenesis

- ♣ Initial vesicular lesion will occur on mouth/face (HSV1) or genitalia (HSV2) _ virus will then travel up the axon and become latent in trigeminal (HSV1) or lumbar and sacral (HSV2) ganglions.
- ♣ Virus can get reactivated by sunlight, hormonal changes, trauma, stress or fever _ after reactivation, virus will travel down the neuron and start replication in the skin and cause vesicular lesions.
- ♣ Vesicles are filled with serous fluid contaminated with virus particles.
- ♣ Can typically find multinucleated cells at the bottom of the vesicles/lesions.
- ♣ Cell mediated immunity is important in limiting herpes virus infections.

Lab diagnosis

- ♣ Causes CPE in cell culture (after 1-3 days)
- ♣ Fluorescent antibody staining
- ♣ Rise in antibody titer can be used to diagnose primary infection but not recurrent
- ♣ Specific glycoproteins with ELISA
- ♣ HSV1 DNA in CSF with PCR (rapid diagnosis)
- ♣ Serological testing with neutralization

Treatment

- **Acyclovir** _ will inhibit viral DNA polymerase and DNA chain termination. Has no effect on latent state!
- Acyclovir is **selectively toxic to alpha herpes viruses because these viruses encode their own thymidine kinase enzymes, which is needed for activation of the drug. Cellular thymidine kinase does not activate the drug.**
- Foscarnet can be used in acyclovir resistant HSV1 strains
- No drug treatment in primary infection prevents recurrences.

Prevention

- Acyclovir can reduce recurrences
- Avoid sun
- Avoid contact with fluid filled vesicles
- C-section for infected pregnant women
- No vaccine available!

Parvoviridae, Poxviridae

POXVIRIDAE

Characteristics

- Is the largest virus
- Linear, double stranded DNA virus, icosahedral (brick shaped), naked.
- Has DNA dependant RNA polymerase (replicates in cytoplasm)
- Consists of several viruses
- **Variola virus** _ smallpox
- **Vaccinia virus** _ is a variant of variola, causes mild disease and is used as the immunogen in smallpox vaccination
- **Molluscum Contagiosum virus** _ causes small wart like lesions on face, arms, back, butt and genitals. It is transmitted by direct contact. It can mimic genital herpes.
- (It is the Variola virus which will be discussed in this title)

Diseases

- Smallpox _ was eradicated by live attenuated vaccine. Last case was in 1977 in Somalia.

Transmission

- By resp. droplets or direct contact with skin lesions.

Pathogenesis

- Infects mucosal cells of upper resp. tract _ spread to local lymph nodes _ then to liver, spleen and later skin through viremia (a condition where virus enters blood system).
- Pharynx _ lymph node _ blood _ organs _ blood _ skin
- Skin lesions will then progress in following way: macule _ papule _ vesicle _ pustule _ crust.

Clinical findings

- After 7-14 days early symptoms and signs of malaise and fever are seen.

Lab diagnosis

- CPE in cell culture or “pocks” on chorioallantoic membrane
- Electron microscopy shows typical particles
- Light microscopy shows cytoplasmic inclusions
- Precipitin test of vesicle fluid will show viral antigens

Treatment

- None

Prevention

- Live attenuated vaccine. It is no longer used, except for maybe in the military.

Mumps

Characteristics

- Belongs to Paramyxoviridae family
- Enveloped, helical, single stranded RNA with negative polarity and has RNA polymerase
- One serotype
- Two types of enveloped spikes
- one with hemaglutinin and neuraminidase activity
- other with F-protein and hemolytic activity
- Its internal nucleocapsid protein is soluble antigens which can be detected in complement fixation (CF) test

Replication

- Penetrates and uncoats _ RNA polymerase transcribes negative strand into multiple mRNA _ mRNA translated into
- proteins _ assembly of nucleocapsids _ released by budding.

Diseases

- Mumps
- Orchitis (inflammation of testis, can cause sterility in men), meningitis, pancreatitis in adult mumps

Transmission

- Respiratory droplets through resp. tract

Pathogenesis

- Infects upper resp. tract _ spread via blood _ parotid gland, ovaries, testis, pancreas, some times meninges
- Maternal antibodies cross placenta and gives protection the first 6 months

Clinical findings

- Incubation time is 18-21 days
- Prodormal stage: fever, malaise, anorexia, followed by swelling of parotid gland
- Resolves itself within 1 week

Lab diagnosis

- Made on clinical grounds
- Virus can be isolated from saliva, urine and CSF and detected by hemadsorption
- Serological testing

Treatment

- No antiviral treatment

Prevention

- **Live attenuated vaccine**, usually given in combination with rubella and measles vaccine to children of 15 months. It is effective, long lasting and with few side effects

Rubella virus

Characteristics

- Belong to Togaviruses
- Enveloped, *icosahedral* in shape, single stranded RNA with *positive* polarity, has surface spikes containing hemaglutinin;
- Since it is positively charged it does not have any virion polymerase!

Replication

- Penetration + uncoating _ the positive strand gets directly translated into structural and non-structural protein _ the *non-structural proteins* will replicate the genome with help of RNA dep. RNA polymerase and make *negative strands* which are used to produce positive stranded progeny, which are then again used to produce more structural/non-structural proteins.
- Replication and assembly takes place in the *cytoplasm* _ virus leaves cell through budding.

Diseases

- Rubella = German measles
- Congenital Rubella Syndrome, can lead to congenital malformations involving the:
    a) Heart (patent ductus arteriosus, septum defects)
    b) Eyes (cataract, glaucoma)
    c) Brain (deafness, mental retardation)

Transmission

- Respiratory droplets
- Transplacentaly

Pathogenesis

- Initial site of infection is nasopharynx, virus will replicate here _ spread to local lymphnodes, replicate _disseminate via blood to skin _ results in rash due to both viral replication and immune injury (Ag-Ab mediated vasculitis).
- In pregnant women, the virus replicates in placenta and spreads to fetus. If it occurs during first trimester it will results in congenital malformations.

Lab diagnosis

- The virus grown in cell culture will interfere will the plaque formation of coxsackievirus, so if there is rubella infection then coxs. will not form plaque (if there is no rubella infection the plaque formation is seen).

Treatment

- None

Prevention

- **Live attenuated vaccine** _ given to kids along with mumps and measles vaccine at 15 months of age.
- Immune serum globulins (IG) are given pregnant women during first trimester to prevent termination of pregnancy.

PICORNAVIRUSES

- Non-enveloped
- Icosahedral nucleocapsid
- Single stranded RNA with positive polarity _ so it will function as mRNA when it enters the cell (no virion polymerase needed)
- Replicated in cytoplasm

2 groups:

- Enteroviruses _ enteric tract infection
- Rhinoviruses _ nose tract infection
- Their optimal temp. is 37 C for Enteroviruses and 33 C for Rhinoviruses
- Enteroviruses are stable in acidic conditions, that's why they can survive in gastric juice.
- Rhinoviruses are acid-labile

Poliomyelitis viruses

Characteristics

- See above
- It has 3 serological types based on antigenic determination and outer capsid proteins

Replication cycle

- Attaches to specific receptors and enter cell _ capsid proteins get resolved _ the RNA genome functions as mRNA and gets translated into one big polyprotein _ this protein gets cleaved by protease into function viral proteins _ these will make negative stranded RNA _ used as template strands _ some of the positive strands function as mRNA to make more viral proteins, the rest will become progeny virion genome RNA _ these are accumulated in the cytoplasm _ virus released upon death/lysis of the cell (not budding!!).

Diseases

- Poliomyelitis (has been eradicated)
- Aseptic meningitis

Transmission

- Through faecal-oral route _ virus will replicate in the ***oropharynx and GI tract***.

Pathogenesis

- Virus will replicate in oropharynx and GI tract _ spread to local lymphnodes _ via blood to CNS, here is will replicate and **damage the motor neurons in anterior horn of spinal cord and brainstem.**
- Damage/death of the motor neurons results in paralysis of muscles innervated by these neurons.
- Damage of brainstem leads to respiratory paralysis (poliomyelitis).
- Immune-response by intestinal IgA and humeral IgG.

Clinical findings

- Incubation: 10-14 days

Response to infection:

1. *Asymptomatic infection*
2. *Abortive poliomyelitis* (most common) _ mild, febrile illness, gives headache, sore throat, nausea, vomiting. Usually spontaneous recovery.
3. *Non-paralytic poliomyelitis* _ aseptic meningitis. Gives fever, headache and stiff neck. Resolves spontaneously.
4. *Paralytic poliomyelitis* _ flaccid paralysis. Brain stem is involved, so it may lead to life threatening paralysis.

Lab diagnosis

- Recovering virus from CSF indicated infection of CNS.
- Isolation of virus from stool indicates infection but not necessarily disease.
- Virus identified in cell culture through CPE.

Treatment

- No antiviral therapy.
- Symptomatic relief and respiratory support if needed.

Prevention

- Both live attenuated and killed vaccine:
- **IPV:** inactivated polio vaccine: killed viruses (type 1, 2 and 3).
- **OPV**: oral polio vaccine: live attenuated viruses (type 1, 2 and 3).

ARBOVIRUSES

- **Arb**ovirus = **AR**thropod – **B**orne – Virus, **meaning these viruses are transmitted by arthropods (e.g. mosquitoes and ticks).**
- In general they are named after the disease that they cause or the place where they were first isolated.

FLAVIVIRUSES (Family of Arboviruses; means yellow)

YELLOW FEVER VIRUS

Characteristics

- Enveloped, single stranded, icosahedral nucleocapsid, positive polarity.
- Causes encephalitis.

Disease

- Fever and jaundice.

Transmission

- By mosquitoes _ infecting humans and birds.
- Occurs in tropical African and South Africa.

It has two epidemiological cycles:

1) Jungle yellow fever :

- reservoir _ monkeys
- vector _ Haemogenous mosquitoes
- hosts _ humans

2) Urban yellow fever:

- reservoir _ humans
- vector _ Aedes aegypti mosquitoes

Pathogenesis and Clinical findings

- It is a severe, life threatening disease
- It starts with sudden onset of fever, headache, myalgia and photophobia. The symptoms will progress to involve kidneys, liver and heart. Prostration (physical collapse) and shock will take place, followed by upper GI hemorrhage with hematemesis.
- Mortality rate is high!

Lab diagnosis

- Isolation of virus
- Rise in Ab titer

Treatment

- No antiviral therapy (that's why mortality rate is high).
- If patient recovers, he/she will have life long immunity.
- No chronic carrier state exists.

Prevention

- **Live attenuated vaccine (17D)** _ protection last for 10 years.

Human tumor viruses (Human papillomaviruses, HTLV-I).

General characteristics:

- Classified either as DNA or RNA virus
- Produce tumors
- Transform infected cells by altering cell growth, cell surface antigens and biochemical processes.
- Introduce "transforming" genes which result in synthesis of one or more transforming proteins.
- **Human tumor viruses include:**

1) HPV
2) HTLV
3) HCV
4) HBV
5) HHV-8
6) **EBV**

HUMAN PAPILLOMAVIRUSES (HPV)

Characteristics

- Belong to Papovaviridae.
- Non enveloped, icosahedral nucleocapsid, circular double stranded DNA, no virion polymerase.
- ~ 60 serotypes _ infect epithelium and cause papillomas at different sites.

Disease

- Papillomas (warts), genital warts associated with cervical and penile cancers.

Transmissions

- Direct contact with skin or genital lesions.

Pathogenesis

- Two viral genes, E6 ad E7, encode proteins that inhibit the activity of proteins that are encoded by tumor suppressor genes (p53 + retinoblastoma). These get inhibited _ leads to cancer.

Lab diagnosis

- Made clinically by finding koilocytes in the lesions. (Koilocyte = squamous cell, often binucleated having a binuclear hole.
- DNA hybridization test are available.
- Virus isolation and serological tests are not done.

Treatment

- Podophyllin or liquid nitrogen most commonly used.
- Alpha-interferons also available.

Prevention

- No drugs or vaccines.

UNIT - 5
PARASITES INFECTIONS

1 PROTOZOON (unicellular)
- Amebas
- Sporozoas
- Flagellates
- Ciliates

2 HELMINTHES (WORMS) (multicellular)

a. PLATHELMINTHES (**Flatworms**)

i. CESTODES (**Tapeworms**)
- *Taenia saginata*
- *Taenia sodium*
- *Diphyllobotrium*
- *Latum*
- *Hymenolepis nana*
- *Echinococci*
- *Dipylidium caninum*

ii. TREMATODES (**Flukes**)
- *Fasciola hepatica*
- *Schistosoma*
- *Paragonimus*
- *Westermani*

b. NEMATHELMINTHES (**Roundworms**)

i. NEMATODES (**Roundworms**)

Intestine nematodes:
- *Enterobius vermicularis*
- *Trichuris trichiura*
- *Ascaris lumbricoides*
- *Toxocara species*
- *Ancylostoma duodenale*
- *Necator americanus*
- *Strongyloides stercoralis*

Tissue infecting filarial nematodes:
- *Wuchereria bancrofti*
- *Loa loa*
- *Onchocerca volvulus*
- *Dracunculus medinensis*

PROTOZOA

A protozoan can be defined as a usually motile eukaryotic unicellular protest and are studied in the discipline called protozoology.
- Unicellular
- Free living
- They have:
  - *Cytoplasmic membrane*
  - *Cellular organs*
  - *Mitochondria*
  - *Food vacuoles*
  - *ER*

- 5 µm – 2 mm in size
- Have an outer *ectoplasm* and an inner *endoplasm*
- They ingest food through their *cystome* (mouth)
- Need Moisture
- Susceptible to desiccation.
- Inhabit freshwater or marine environments.
- Many terrestrial protozoa can be found in decaying organic matter, in soil, and even in beach sand;
- Some are parasitic in plants or animals.
- Chemoheterotrophic
- In Encystations, they develop into a resting stage called a cyst,
- Cysts serve three major functions:
    - Protection
    - Nuclear reorganization and cell division
    - Transfer between hosts in parasitic species.
- In excystation (escape from the cysts) is unknown
- Excystation generally is triggered by a return to favourable environmental conditions.
- For example, cysts of parasitic species excyst after ingestion by the host and form the vegetative form called the trophozoite.
- Three major types of locomotory organelles: pseudopodia, flagella, or cilia.
- A few protozoa are nonmotile.
- Most protozoa reproduce asexually, and some also carry out sexual reproduction. The most common method of asexual reproduction is binary fission.
- They reproduce:
    - **Asexually** – by DNA replication followed by division into 2 cells
    - **Sexually** – by fusion of 2 cells, exchange of material and then division into 2 cells again
- When they enter a new environment, they shrink into a **cyst** – it is in this form that they becomeinfective to people who ingest it. After ingestion they transform back into the motile form, **trophozoite**.

Classification of Protozoa:

1. Amebas (Sarcodina)

a. Entamoeba
b. Histolytica
c. Free living Amebas

2. Flagellatas (Mastigophora)

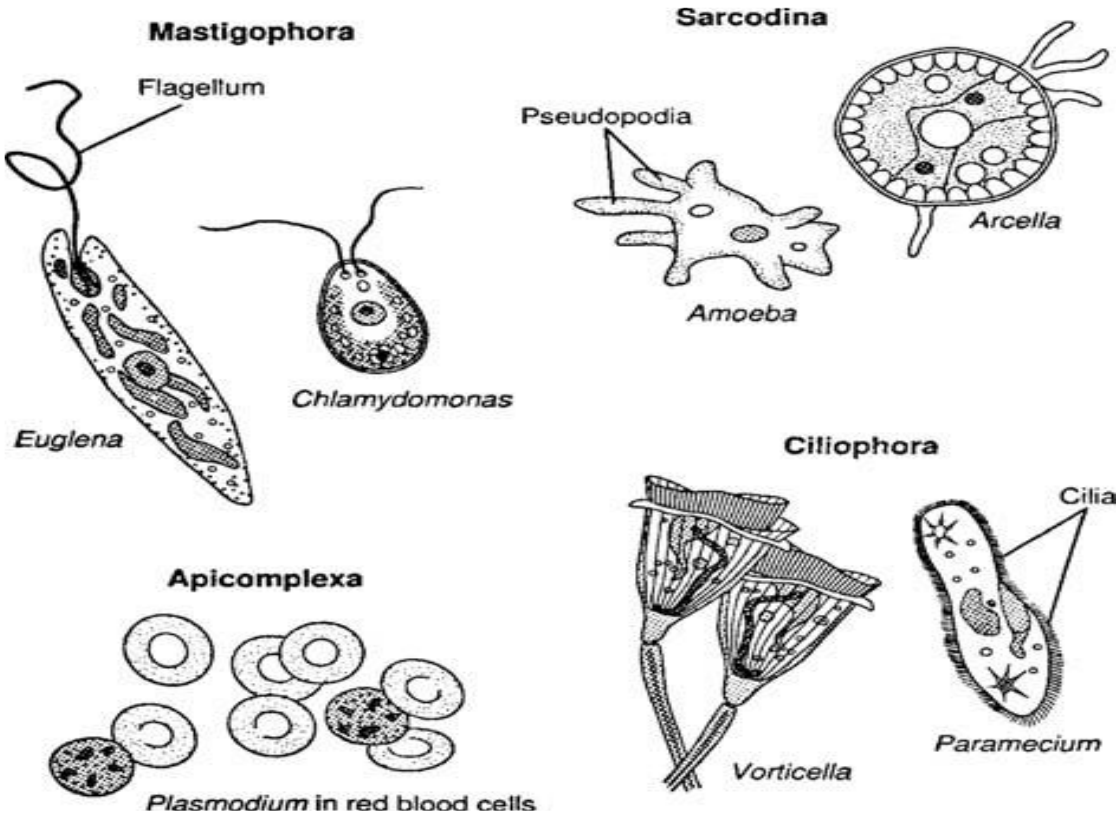
d. Giardia lamblia
e. Trichomonas
f. Vaginalis
g. Trypanosoma
h. Leishmania

3. Ciliata (Ciliophora)

i. Balantidium coli

4. Sporozoa (Apicomplexa)

a. Plasmodium
b. Toxoplasma gondii



NEMATODES (ROUNDWORMS)

- Non-segmented roundworms with cylindrical bodies.
- Have complete digestive system including mouth and anus.
- Covered by a non-cellular, highly resistant coat called **cuticle**.
- Both male and female
    - Female is larger
    - Male has a coiled tail

Medically important nematodes are divided into 2 categories:

1. Intestinal Nematodes

1. Enterobius
2. Trichuris Transmitted by ingestion of *egg*.
3. Ascaris
4. Necator
5. Strongyloides
6. Toxocara Transmitted by ingestion of *larvae*.
7. Trichinella
8. Ancylostoma

2. Tissue Nematodes

1. Wuchereria Also called "**Filarial Worms**" because they produce motile
2. Loa embryo called **microfilaria** in blood and tissue.
3. Onchocerca Transmitted by *mosquitoes* or *flies*.
4. Dracunculus

Trematodes (Flukes)

- Are *flat, elongated* and *leaf shaped*.

- They are all **hermaphroditic**, except for Schistosoma.
- They have unique sexual cycles:
  - *Sexual* reproduction occurs in *vertebral host* (e.g. humans).
  - *Asexual* reproduction occurs in *snails* (intermediate hosts).
- Transmission to humans happens either by:
  - Penetration of skin by free swimming cercaria of Schistosomas.
  - Ingestion of cysts in undercooked/raw meat or crab in Fasciola and Paragonimus.

Intestinal amebas

Entamoeba histolytica

Characteristics

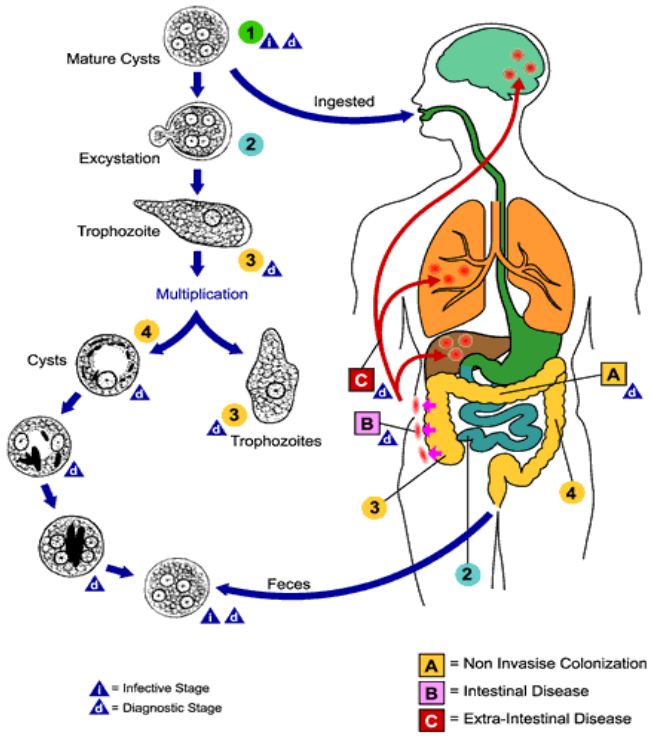
- Intestinal protozoa, free living, motile ameba (throphozoite).
- Has 2 life cycles:
  - Motile ameba (trophozoite).
  - Non-motile cyst.
- The cyst has *4 nuclei* _ important in diagnosis.
- The cysts are not very resistant and can easily be killed by *boiling* (not by chlorination of water!)

Transmission

- *Fecal-oral* route by ingestions of cyst in contaminated food/water or *anal-oral* transmission.
- Humans are the only reservoirs (no animal reservoir).
- Histolytica is found worldwide, but occurs most frequently in tropical countries (especially in areas with *poor sanitation*).

Disease

- Amebic dysenteric and liver abscess.



Life cycle

- Ingested cyst will differentiate into trophozoite in ileum, but tends to colonize cecum and colon.
- Trophozoite invades the intestinal epithelium and secretes enzymes that will cause local necrosis (lesions).
- As the lesions reach the muscularis layer, a typical teardrop ulcer forms which can destroy large areas of epithelium.
- The ulcer will make its way down to submucosa, which leads to invasion of portal circulation by the trophozoites resulting in systemic infection in liver _ **amebic abscess**

Clinical findings

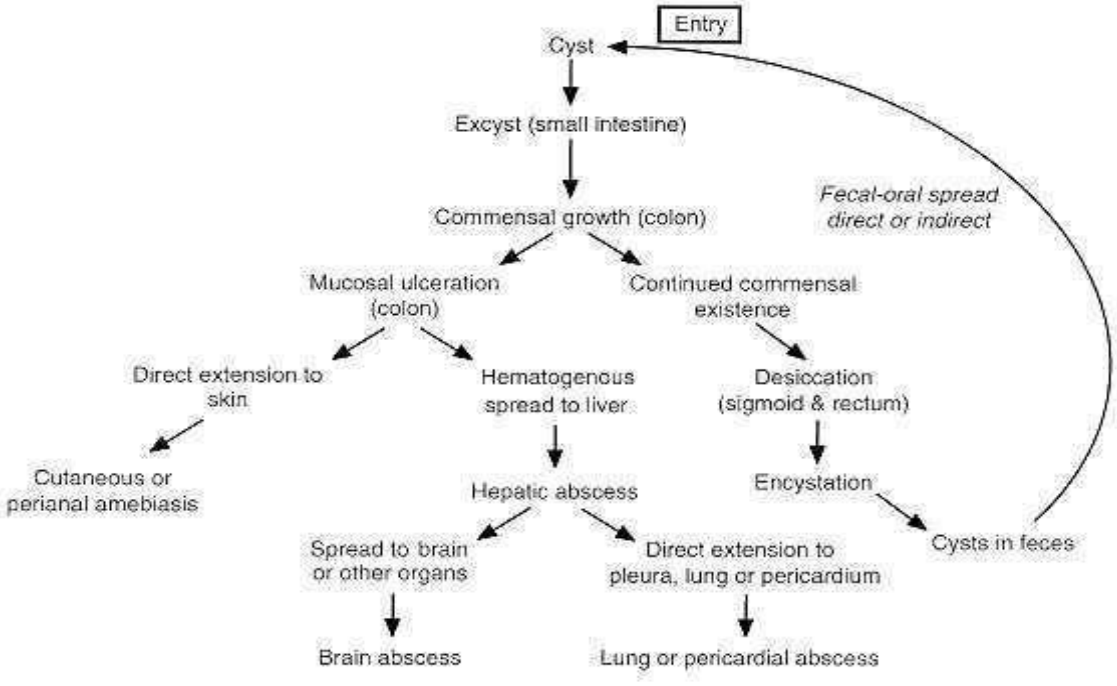
- **Dysentery: bloody mucous containing diarrhea.**
- Lower abdominal pain, flatulence and **tenesmus** (feeling of incomplete defecation, improper emptying).
- ~90% of those infected are asymptomatic carriers and they may infect others as the cyst is passed in feces.
- Amebic abscess of liver is characterized by right-upper quadrant pain, weight loss, fever
- and tender enlarged liver.

Complications might be:

- **Amoeboma** _ tumor like enlargement of small intestine and
- **Skin amebiasis** – in perianal region.

Lab diagnosis

- Based on finding *trophozoites in diarrhea stool* or *cyst* (with 4 nuclei) in *formed stool* ~1 hour after collection.
- Trophozoites characteristically contain *ingest RBC* (important to not mistake them for fecal leukocytes).
- *E. histolytica* has to be distinguished from *Entamoeba coli* – which is part of normal flora, distinction is made based on:
    - **Cyst size and number of nuclei:** E. Histolytica cyst is *small with 4 nuclei*, Entamoeba Coli cyst are *larger with 8 nuclei*.
    - **Presence or absence of RBC in trophozoites**: E. Histolytica has, Entamoeba Coli doesn't.
- Complete examination of cyst involves: wet mount in saline, iodine-stained wet mount and a fixed trichome stained preparation.



- Serologic testing – indirect hemagglutination.

Treatment

- Metronidazole

Prevention

- Good personal hygiene – wash hands
- Proper disposal of human waste
- Water purification

Malaria

- ***Plasmodium* species as a causative agent**.

Plasmodium genus:

1. ***P. vivax***
2. ***P. ovale***
3. ***P. malariae***
4. ***P. falciporum***

Characteristics

- They are protozoa that infect RBC and tissues (liver, kidney, brain).

Transmission

- By **female Anophele mosquito.**

Epidemiology

- Occurs in tropical areas of Asia, Africa and Latin America.

Life cycle

- Their life cycle has 2 phases:
  1. **Sexual/Sporogenic Cycle** – consists of **gametogony** (formation of **gametes**) in humans and **sporogony** (formation of **sporozoites**) in the mosquitoes.
  2. **Asexual Cycle** – consist of **schizogony** (formation of **schizonts**) and takes place in humans.

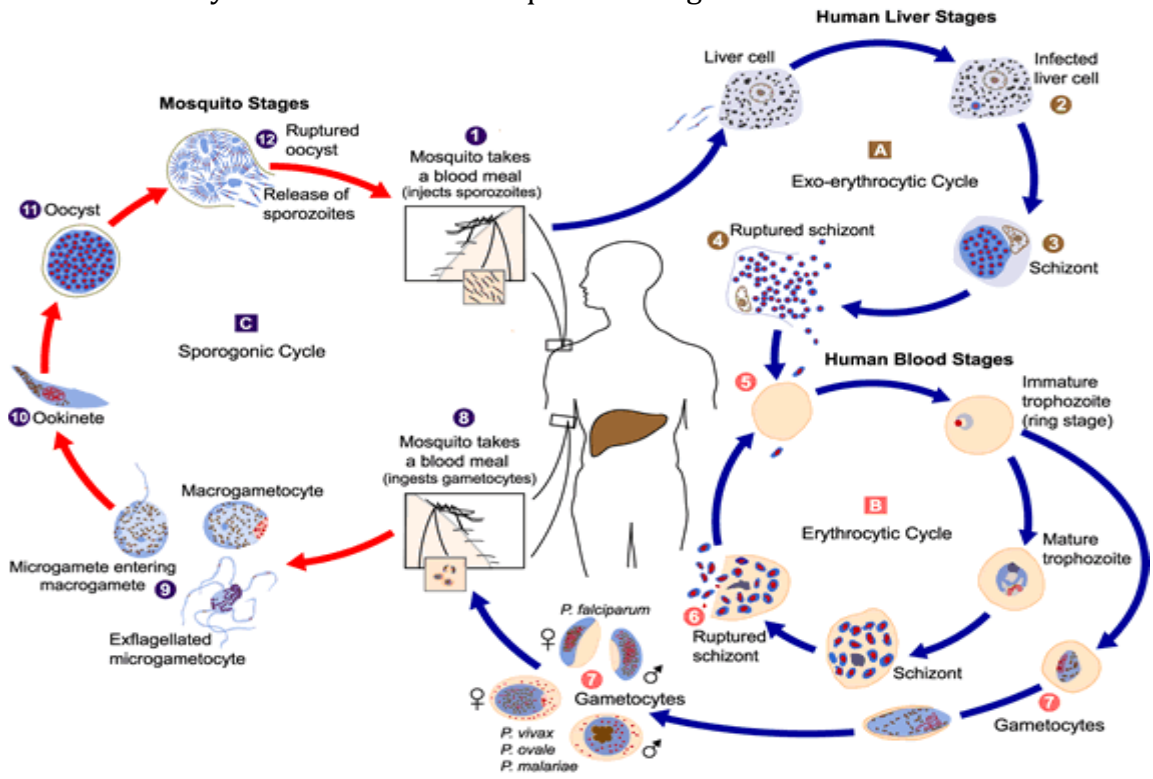
Asexual Cycle

- The Asexual cycle starts with the female Anophele mosquito biting the human and like this introducing the **sporozoites** from its saliva into the human blood stream.
- The sporozoites will via the blood stream invade the *hepatocytes* of the liver = **Exoerythrocytic Phase**. In the liver they multiply and differentiate into **merozoits** (in this stage *P. vivax* and *P. ovale* produce merozoits but a *latent* form called **hypnozoits** instead, which is the cause of the *relapse* seen in P. vivax/ovale Malaria).
- Merozoits will leave the hepatocytes and infect RBC = **Erythrocytic Phase**. In the RBC, the plasmodium will differ into a ring shaped throphozoite called **schizont** which will release more merozoits which will in turn infect more erythrocytes in a synchronized way – this periodic release of merozoits is what causes the typical recurring symptoms of chills, fever and sweating see in malaria patients!! The Erythrocytic cycle **takes 48h for *P. Vivax* and *P. ovale* and 72H for *P. malariae*.**

Sexual Cycle

- The Sexual cycle starts in the human erythrocyte where some of the merozoits develop into male and female **gametocytes**.

- These are then taken up by the female Anophele mosquito (when it bites) and inside her gut the gametocytes will release female and male **gametes**.
- The gametes will unite to form a **zygote** which will in turn for an **oocyst** containing many sporozoites.
- The sporozoites will be released and migrate to the mosquitoes salivary gland where they remain until the mosquito bites again.



Pathogenesis

- Malaria is subdivided into:
  - **Benign Malaria** _ *P. vivax* and *P. ovale*.
- Disease caused by *P vivax* and *P. ovale* are characterized by typical paroxysm occurring *~3 days* and are thus called **Tertian Malaria** (aka Benign Tertian Malaria)
  - **Malignant Malaria** _ *P. falciporum*.
- Diseases by *P. falciporum* characteristically occur every 4 days and therefore called **Quartian Malaria**.
- Most pathological findings are due to the destruction of RBC by the merozoits.
- *P. falciporum* causes the most severe malaria because it *infects allot more RBC* and causes aggregation of RBC which occlude capillaries _ will cause anoxia of tissue leading to necrosis:
  - Brain - Cerebral Malaria
  - Kidneys - hematuria _ dark colored pee ("black water fever").
- *P. falciporum* can produce a much higher level of parasite count because it can infect RBC of all ages; while *P. vivax* only infects reticulocytes and *P. malaria* only mature RBC giving them a much lower parasite count.
- People with sickle cell anemia are protected against malaria because their RBCs have too little ATPase activity to support the growth or parasites.
- Malaria can also be transmitted across placenta, via blood transfusions and intravenous drug use.

- More than 200 million people are infected with it and more than 1 million die each year most common lethal disease.

Clinical findings

- Incubation period *~2 weeks* – then stats fever, chills, headache and myalgia.
- Periodic fever starts a few days after this and can reach up to 41C followed by nausea, abdominal pain and drenching sweat.
- Splenomegaly, anemia and hepatomegaly in 1/3.
- Relapse of *P. vivax* and *P. ovale* can occur several *years* after initial incident cause of latent hypnozoits in the liver.
- Malaria caused by *P.falciporum* can be fatal if untreated.

Lab diagnosis

- Giemsa stained blood smear. Thick smear used to screen for the agent, thin smear used to identify the species.
- It is important to know the species as the treatment is different for each.
- Trophozoites – ring shaped.
- Gametocytes of *P.falciporum* – banana shaped.
- All the other are spherical.

Treatment

- Chloroquine for acute malaria _ kills merozoits and reduces parasitemia.
- Primaquine kills hypnozoits _ prevents relapse.
- Mefloquine for chloroquine resistant strains.

Prevention

- Chemoprophylaxis for travelers for 2 weeks before arrival and 6 weeks after departure from country – consist of mefloquine and chloroquine.
- Protection against bite (clothing, net etc).

ASCARIS LUMBRICOIDES

Disease

- **Ascariasis**.

Characteristics

- Is a geohelminth.
- Largest roundworm ~20-35 cm.

Life cycle

a. Humans ingest the *embryonated/infective eggs* which hatch in the small intestines.
b. The larvae migrate through the gut wall into blood vessels where they are carried by the blood stream to the *lungs*.
c. The larvae mature further in the lungs (*10-14 days*) _penetrate the alveoli and bronchi ascend up the trachea until they reach the *throat* and are then *swallowed*.
d. When they reach the small intestine, they develop into adult worms – they get their nutrition from ingested food.
e. The adult female produces *thousands* (200 000) of infertile eggs *daily* that are passed in feces.

f. The fertile eggs develop en embryo and become infective in 18 days to several weeks after being passed in human feces, depending on the environmental conditions (optimum: *moist, warm, shaded soil*). The eggs are very resistant environmental conditions.

- It takes about *2-3 months* from swallowing an infective egg to develop mature adult worms.
- Adult worms can live *1-2 years* inside the gut.

Transmission and Epidemiology

- Transmitted by eating food etc contaminated eggs (via soil/feces).
- Humans are the only hosts.
- Endemic in tropical areas.

Clinical findings and Pathogenesis

- The larvae migration causes the most damage, especially in the lungs:
  - Inflammation with eosinophilic exudates in response to larvae antigens.
  - Pneumonia (Loeffler's pneumonia) can occur with dyspnea, dry cough and fever.
  - Malnutrition can occur, especially in children in developing countries since adult worms feed off of ingested food.
- Obstruction of small intestines dude to mass of tangled worms.
- **Autoreinfection cannot occur, because the eggs mature in the soil and it takes several days before they become infectious (embryonated).**

Lab diagnosis

- Detection of *eggs in stool – oval, irregular in shape.*
- Sometimes adult worms are also passed in stool and can be visualized.

Treatment

- Thiabendazole – for during lung migrations.
- Mebendazole for after.

Prevention

- Proper disposal of feces.

WUCHERERIA BANCROFTI

Disease

- **Filariasis**
- **Elephantiasis** _ chronic condition

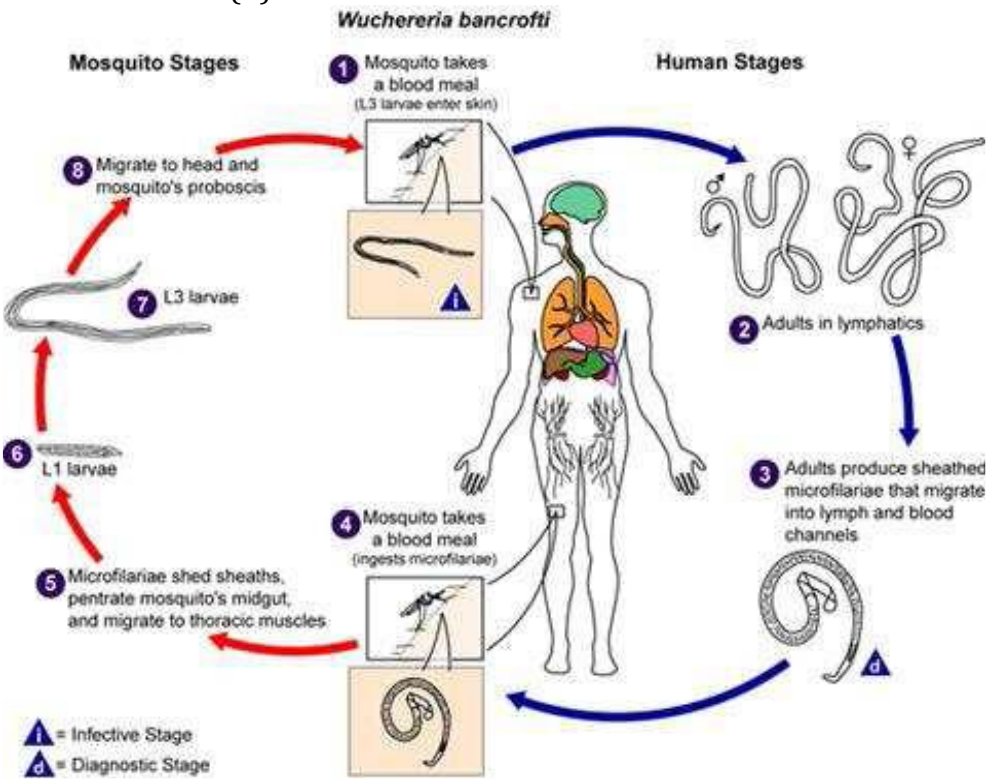
Characteristics

- Male ~*4cm*
- Female ~*9 cm*
- Called filarial because they give rise to motile embryo –*microfilaria (*244-296 µm).

Life cycle

a. During a blood meal, an infected mosquito introduces third-stage filarial larvae onto the skin of the human host, where they penetrate into the bite wound (**1**).

b. They develop in adults that commonly reside in the lymphatics (**2**).
c. The adults produce **microfilaria** measuring which are *sheathed* and have *nocturnal periodicity*. The microfilarias migrate into lymph and blood channels moving actively through the vessels (**3**).
d. A mosquito ingests the microfilaria during a blood meal (**4**).
e. After ingestion, the microfilarias lose their sheaths and some of them work their way into the thoracic muscles (**5**).
f. Here the microfilarias develop into **first-stage larvae** (**6**) and subsequently into **third-stage infective larvae** (**7**).
g. The third-stage infective larvae can infect another human when the mosquito takes a blood meal (**1**).



Transmission & Epidemiology

- Transmitted through bite of *female mosquito* – larvae deposited – penetrates skin.
- **Vector** – different *mosquitoes* (depending on the parts of the world).
- Humans are the *only definitive host*.
- Adult worms can live for many years inside the humans.
- ~200-300 people are infected.

Clinical finding & Pathogenesis

- Adult worms in lymph nodes cause inflammation which eventually obstructs the lymphatic vessels _ cause **edema**.
- The edema is soft at first but becomes fibrotic _ skin will crack and can become disposed to secondary infection by pyogenic/mycotic organisms.
- **Elephantiasis** occurs mainly in patients who are repeatedly infected over a long period of time _ chronic state (1%).
- **Tropical pulmonary eosinophilia** (Weingarten's syndrome) with nocturnal cough, breathlessness and wheezing are characteristics for infection.

Lab diagnosis

- Thick blood smear with blood sample taken from patient during night will show microfilaria. They are also sometimes seen in urine. They can't be seen during incubation period, only during acute phase.
- Serology not useful.

Treatment

- Surgical removal of excess elephantoid tissue (breast, scrotum, legs, stomach).
- Diethylcarbamazine.

Prevention

- Mosquito control with insecticides, net, repellents and protective clothing.

Giardia lamblia

Characteristics

- Belongs to flagellata group.
- Has 2 life cycles:
    - **Pear shaped trophozoite** with *2 nuclei*, *4 flagella* and a *suction cup* which it uses to attach to the intestinal with.
    - **Oval cyst** with *4 nuclei* _ gives rise to *2 trophozoites*.

Disease

Giardiasis

Transmission and Epidemiology

- Through fecal-oral route _ fecal (cyst) contaminated food/water.
- Giardiasis is found worldwide.
- ~50% of those infected are asymptomatic carriers _ excrete the cyst for years.
- Those who are IgA deficient are predisposed to symptomatic infection.
- Incidences are high among children in daycares, patients in mental hospitals, male homosexuals (oral-anal) and hikers who drink untreated stream water.
- Boiling will kill the cyst, chlorination will not.

Life cycle

a. Ingestions of cyst _ throphozoite will form in the duodenum.
b. The throphozoite with attach to intestinal wall, but wont invade it.
c. It will cause inflammation of mucosa leading to malabsorption of proteins and fat.

Clinical findings

- Non bloody, foul smelling, fatty diarrhea (fatty because fat is not absorbed) – this is followed by nausea, anorexia, flatulence and abdominal cramps persisting for weeks to months. No fever.
- Because fat is not absorbed, there can be a loss of fat-soluble vitamins D, E, K & A.

Lab diagnosis

- Trophozoites/cysts can be observed in diarrhea/formed stool (cyst in formed stool is seen in asymptomatic carriers).
- If microscopic test is negative, **String test** is performed _patient swallows a string with a weight attached – the string will reached the duodenum – trophozoites will attach to it and can be visualized afterwards.
- No serology test.

Treatment

- Metronidazole.

Prevention

- In endemic areas _ drink only boiled, filtered or iodine treated water.
- Proper hygiene (hand wash).

Trichomonas vaginalis

Characteristics

- ✓ Belong to flagellata group and is a *urogenital protozoan*.
- ✓ It is *pear shaped* with *4 flagella* and has an *undulating membrane* that extends until 2/3 of its length.
- ✓ Found only in trophozoite form. *NO CYST!*
- ✓ Very common, found worldwide.

Disease

Trichomoniasis (STD)

Transmission

- ✓ Via *sexual contact* – mostly found residing in vagina and prostate.
- ✓ Predisposing factors to symptomatic infection is loss of normal acidity of vagina.
- ✓ ~25-50% of population harbors the organism (very common).

Clinical findings

- ✓ In **women** _ watery, foul smelling greenish discharge accompanied with itching and burning. If the parasite reaches other parts it might cause **cervicitis** (depends on normal flora and pH of vagina).
- ✓ In **males** _ usually asymptomatic, but 10% of cases causes **urethritis**.
- ✓ The parasite can also carry other organisms e.g. candida.

Lab diagnosis

- ✓ Direct demonstration of protozoa in wet mount or stained smear of clinical specimen (vaginal, urethral, prostatic secretion).

Treatment

- ✓ Metronidazole

Prevention

- ✓ Condoms
- ✓ No drug or vaccine available.

Leishmania donovani

Characteristics

- ŏ Is a blood and tissue flagellata protozoa.

Vector:

- ŏ **Sandfly** – only the females can transmit disease.

Reservoir:

- ŏ dogs,
- ŏ foxes,
- ŏ rodents
- ŏ human reservoirs in India

Disease:

- ŏ **Kala-azar** (*Visceral Leishmaniasis – black sickness*).
- ŏ Disease is mainly found in: Africa, Middle East and parts of China.

Life cycle

a. Human *macrophages* containing *amastigotes* are ingested by the Sandfly.

b. In the fly gut they differentiate into **promastigotes** then migrate to the *pharynx* of the fly.

c. When the fly bites again – the promastigotes enter macrophages in the host's blood and transform into *amastigotes*.

d. The macrophages will die and release the amastigotes which will infect other macrophages and reticuloendothelial cells (especially in the *liver*, *spleen* and *bone marrow*).

Pathogenesis

- ŏ Amastigotes kill reticuloendothelial cells, especially in liver, spleen and bone marrow. Destruction of spleen leads to anemia, leukopenia and thrombocytopenia which can lead to secondary infections.

Clinical findings

- ŏ Starts with intermittent fever, weakness and weight loss.
- ŏ Splenomegaly
- ŏ Hyperpigmentation of skin – seen in light skinned patients, hence the name "black sickness"
- ŏ Can be fatal if untreated, mortality ~5%.

Lab diagnosis

- ŏ Amastigotes visible in smear sample taken from spleen, bone marrow or lymph nodes.
- ŏ Culture.
- ŏ Serology (immunofluorescence).

Treatment

- ŏ Sodium-stibogluconate.

Prevention

- ŏ Protection from bite.
- ŏ No vaccine.

Taenia solium

Disease

Taeniasis and **Cysticercosis**.

Characteristics

- Ψ Pork tapeworm – adult get ~5m long!
- Ψ Scolex has 5 suckers and a circle of hooks.
- Ψ Their gravid proglottid has 5-10 primary uterine branches. a) T. Solium b) T. Saginata

Life cycle:

a. Pigs accidentally eat gravid proglottids containing eggs – in the pig gut embryo with 6 hooks (**oncospheres**) will hatch from the eggs.

b. The embryo burrows themselves into a blood vessels and like this carried to skeletal muscle, here they develop into **cysticerci larvae** and reside here until ingested by host.

c. Humans ingest undercook/raw pork containing **cysticerci larvae** (**cysticercus cellulosa**).

d. In the small intestine the larvae attach to gut wall and grows into adult worm with gravid proglottids (~3 months).

e. The gravid proglottids detachdaily and are passed in feces.

If humans ingest food contaminated with *T. Sodium* **eggs**, the eggs will hatch in small intestines _ the oncospheres embryo will burrow into blood vessel and get disseminated to various organs, especially **eyes** and **brains** where they encyst and form cysticerci _ **Cysticercosis** (causes major damage!).

Transmission & Epidemiology

- Ψ Taeniasis acquired by eating raw/undercooked pork.
- Ψ Cysticercosis acquired only by eating eggs in fecal contaminated food/water.

- Occurs worldwide – endemic in Asia, Latin America and Easter Europe.
- **Intermediate hosts**: pigs or humans
- **Definitive hosts:** humans

Clinical findings & Pathogenesis

- Adult tapeworm in gut causes little damage – most asymptomatic (some experience diarrhea, abdominal pain and weight loss).
- Cysticerci however can get very large and cause mass lesions in:
- **Brain** _ manifested as space occupying lesions (headache, vomiting and seizures).
- **Eyes** _ can appear as uveitis and retinitis. Larvae can also be observes floating around in vitreous body.
- Living cysticerci doesn't cause inflammation, but when they die they release a substance which causes inflammatory response.
- Eventually the cysticerci calcify – can be seen on X-ray.

Lab diagnosis

- Demonstration of characteristic proglottids and eggs in feces (eggs less commonly found).
- Cysticercosis diagnosis by demonstrating cyst in tissue by X-ray or CT-scan.

Treatment

- Niclosamide _ for adult worms.
- Praziquantel (or Albendazole) _ for cysticercosis, although surgical removal might be required.

Prevention

- Cooking pork adequately.
- Prevent human waste being dumped around pig bins.
- Proper hygiene (hand wash).

Ancylostoma duodenale

Disease

Hookworm

- Ancylostoma Duodenale _ *Old World Hookworm*
- Necator Americanus _ *New World Hookworm*

Characteristics

- Are geohelminths.
- Adults are ~1 cm long.
- Ancylostoma Duodenale has teeth.
- Necator Americanus has cutting plates.

Life cycle

a. Eggs are passed in the stool, and under favourable conditions (moisture, warmth, shade), larvae hatch in 1- 2 days.
b. The released **rhabditiform** larvae grow in the feces and/or the soil, and after 5 to 10 days they become **filariform** larvae that are infective. These infective larvae can survive 3 to 4 weeks in favourable environmental conditions.
c. On contact with the human host, the larvae penetrate the skin and are carried through the veins to the heart and then to the lungs. They penetrate into the alveoli, ascend the bronchial tree to the pharynx, and are swallowed.
d. The larvae reach the small intestine, where they reside and mature into adults.
e. Adult worms live in the lumen of the small intestine, where they attach to the intestinal wall and result in blood loss by the host.

- ♣ Most adult worms are eliminated in 1 to 2 years, but some can stay alive for up to 10 years.
- ♣ Some A. Duodenale larvae, following penetration of the host skin, can become dormant (in the intestine or muscle).
- ♣ Infection by A. Duodenale may also occur by the oral and transmammary route. N. Americanus, however, requires a transpulmonary migration phase.

Transmission & Epidemiology

- ♣ Transmission - filariform larvae in soil penetrates the skin on feet.
- ♣ Found worldwide – especially in tropical areas (endemic).
- ♣ ~700-900 million are infected.
- ♣ Humans are the only hosts.

Clinical findings & Pathogenesis

- ♣ Incubation period ~6 weeks.
- ♣ Main damage is dude to blood loss, ~0.1-.03ml blood can be lost per day per worm _ can lead to **anemia**. (The worms also produce and anticoagulant to keep blood flowing).
- ♣ **Ground itch**, papule/vesicle, burning sensation and edema at site of larvae entry.
- ♣ **Pneumonia with eosinophilia** – due to larvae migration to lung.
- ♣ In areas where hookworm is endemic, chronic hookworm disease is most characteristics: anemia, epigastric burning, flatulence, tender abdomen, alternating diarrhea/constipation, dry skin, blurred vision, **cardiomegaly** and **cardiac symptoms** in many cases (can lead to death due to hear failure).

Lab diagnosis

- ♣ Observing *eggs in stool* microscopically.
- ♣ Blood in stool + eosinophilia is common (eosinophilic granules in blood).
- ♣ Decreased hemoglobin and serum iron levels are common.
- ♣ Serology not useful.

Treatment

- ♣ Mebendazole – it is hard to eradicate because reinfection usually occurs.
- ♣ Iron supplement can be helpful.

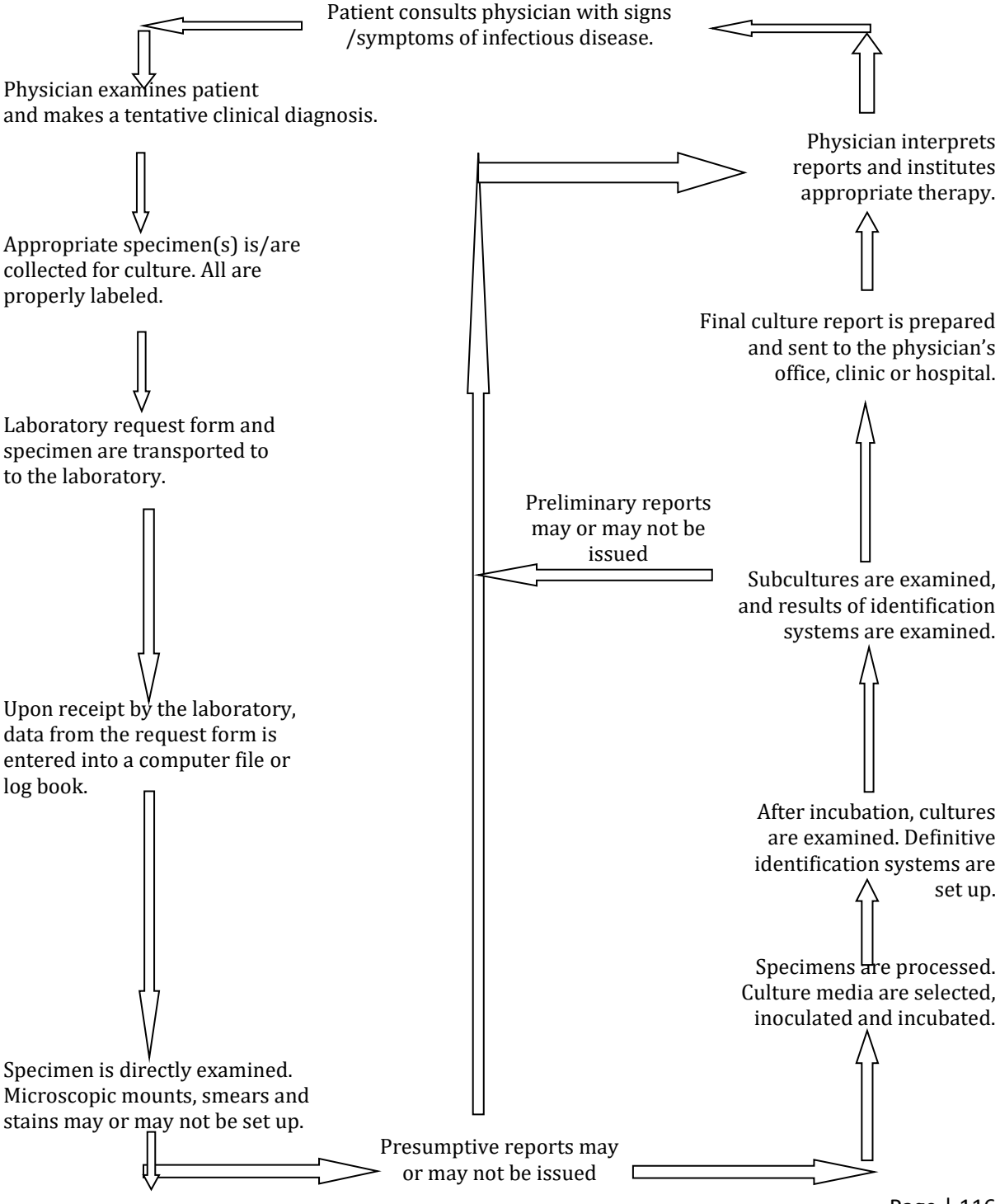
Prevention

- ♣ Proper disposal of sewage.
- ♣ Protection of feet/legs.

A Schematic Overview of the Diagnostic Cycle

Diagnostic medical microbiology is concerned with the etiologic diagnosis of infection. Laboratory procedures used in the diagnosis of infectious disease in humans include the following:

1. Morphologic identification of the agent in stains of specimens or sections of tissues (light and electron microscopy). Culture isolation and identification of the agent.
2. Detection of antigen from the agent by immunologic assay (latex agglutination, enzyme immunoassay [EIA], etc) or by fluorescein - labelled (or peroxidase-labelled) antibody stains.
3. DNA-DNA or DNA-RNA hybridization to detect pathogen specific genes in patients' specimens. Detection and amplification of organism nucleic acid in patients' specimens.
4. Demonstration of meaningful antibody or cell-mediated immune responses to an infectious agent.



DIAGNOSIS OF BACTERIAL INFECTIONS

Specimens

Any tissue or body fluid can be subjected to microbiological investigation with the aim of identifying the infecting pathogen and predicting response to therapy.

To optimize the diagnostic benefit it is necessary to:

- understand in which tissues/specimens the organism is to be found and when in the natural history of the infection;
- take samples carefully (e.g. poor aseptic technique may lead to contamination of sterile samples causing false-positive results);
- transport samples rapidly to the laboratory in a suitable medium.

It is important to remember that some organisms survive poorly outside the body (e.g. numbers of strict anaerobes are reduced by atmospheric oxygen) and in some cases suitable media needs to be inoculated directly in the clinic (e.g. for isolation of *Neisseria gonorrhoeae*).

Eg:- blood, body fluid, tissue or faeces to aid diagnosis.

A laboratory request form with the following information must accompany the specimen.

- Patient's name, DOB, ward/department and hospital number
- Type of specimen and the site from which it was obtained
- Date and time collected
- Diagnosis with history and reasons for request such as returning from abroad (specify country) with diarrhoea and vomiting, rash, pyrexia, catheters in situ or invasive devices used, or surgical details regarding postoperative wound infection
- Any antimicrobial drug(s) given
- Consultant's name
- Name/bleep number of the clinician who ordered the investigation, as it may be necessary to telephone preliminary results and discuss treatment before the final result is authorised

Blood specimen collection

- Place a tourniquet above the venepuncture site. Disinfect the tops of blood culture bottles.
- Palpate and locate the vein. It is critical to disinfect the venepuncture site meticulously with 10% povidone iodine or 70% isopropyl alcohol by swabbing the skin concentrically from the centre of the venipuncture site outwards. Let the disinfectant evaporate. Do not repalpate the vein again. Perform venipuncture.
- If withdrawing with conventional disposable syringes, withdraw 5-10 ml of whole blood from adults, 2-5ml from children and 0.5-2ml for infants. Using aseptic technique, transfer the specimen to relevant cap transport tubes and culture bottles. Secure caps tightly.
- If withdrawing with vacuum systems, withdraw the desired amount of blood directly into each transport tube and culture bottle.
- Remove the tourniquet. Apply pressure to site until bleeding stops, and apply bandaid.
- Label the tube, including the unique patient identification number, using indelible marker pen.
- Do not recap used sharps. Discard directly into the sharps disposal container.

- Complete the case investigation and the laboratory request forms using the same identification number.

Cerebrospinal fluid

- As only experienced personnel should be involved in the collection of CSF samples, the method is not described in this document.
- CSF is collected directly into the separate screw-cap tubes. If the samples will not be promptly transported, separate tubes should be collected for bacterial processing.

Method of collecting a stool

- specimen Collect freshly passed stool, 5 ml liquid or 5 g solid (pea-size), in a container.
- Label the container.

Method of collecting a rectal swab from infants

- Moisten a swab in sterile saline.
- Insert the swab tip just past the anal sphincter and rotate gently.
- Withdraw the swab and examine to ensure that the cotton tip is stained with faeces.
- Place the swab in sterile tube/container containing the appropriate transport medium.
- Break off the top part of the stick without touching the tube and tighten the screw cap firmly.
- Label the specimen tube.

Method of collecting a throat swab

- Hold the tongue down with the depressor. Use a strong light source to locate areas of inflammation and exudate in the posterior pharynx and the tonsillar region of the throat behind the uvula.
- Rub the area back and forth with a Dacron or calcium alginate swab. Withdraw the swab without touching cheeks, teeth or gums and insert into a screw cap tube containing transport medium.
- Break off the top part of the stick without touching the tube and tighten the screw cap firmly.
- Label the specimen containers.
- Complete the laboratory request form.

Method of collecting nasopharyngeal swabs (for suspected pertussis)

- Seat the patient comfortably, tilt the head back and insert the nasal speculum.
- Insert a flexible calcium alginate/Dacron swab through the speculum parallel to the floor of nose without pointing upwards. Alternately, bend the wire and insert it into the throat and move the swab upwards into the nasopharyngeal space.
- Rotate the swab on the nasopharyngeal membrane a few times, remove it carefully and insert it into a screw cap tube containing transport medium.
- Break off the top part of the stick without touching the tube and tighten the screw cap firmly.
- Label the specimen tube, indicating left or right side.
- Complete the laboratory request form.
- Repeat on the other side.

Method of collecting sputum

- Instruct patient to take a deep breath and cough up sputum directly into a wide-mouth sterile container. Avoid saliva or postnasal discharge. Minimum volume should be about 1 ml. Label the specimen containers.
- Complete the laboratory request form.

Urine specimen

- Labels and indelible marker pen.
- Give the patient clear instructions to pass urine for a few seconds, and then to hold the cup in the urine stream for a few seconds to catch a mid-stream urine sample. This should decrease the risk of contamination from organisms living in the urethra.
- To decrease the risk of contamination from skin organisms, the patient should be directed to avoid touching the inside or rim of the plastic cup with the skin of the hands, legs or external genitalia. Tighten the cap firmly when finished.
- For hospitalized or debilitated patients, it may be necessary to wash the external genitalia with soapy water to reduce the risk of contamination. If soap and clean water are not available, the area may be rinsed with normal saline. Dry the area thoroughly with gauze pads before collecting the urine.
- Urine collection bags may be necessary for infants. If used, transfer urine from the urine bag to specimen containers as soon as possible to prevent contamination with skin bacteria. Use a disposable transfer pipette to transfer the urine.
- Label the specimen containers.

Transport to the laboratory:

- Charcoal medium improves the isolation of bacteria by neutralising toxic substances such as naturally occurring fatty acids found on the skin.
- Amies Bacterial Transport Medium
- Anaerobic Bacterial Transport Medium
- Fecal Transport Medium
- Transport Medium
- Cary and Blair Medium
- Stuarts medium
- Venkatraman Ramakrishnan (VR) medium

Equipment

This will vary according to the specimen required but must include:

- disposable gloves
- additional personal protective equipment (PPE) where applicable (eg apron/gown, respirator, visor)
- a plastic tray
- a sterile container for the specimen
- appropriate transport medium, if required
- laboratory specimen form
- a polythene transportation bag
- biohazard label, if required.

Laboratory examination

Specimens may be examined grossly, for example to detect adult worms in faeces. Although microscopy is rapid, it is insensitive and requires considerable expertise; specificity may also be a problem if commensal organisms can be mistaken for

pathogens. Microscopy can also be used to define specimen quality, for example identifying salivary contamination of sputum by the presence of epithelial cells.

Special stains can be used to help identify organisms, such as Giemsa staining of blood films and tissues, which is used to identify malaria and Leishmania. Immunofluorescence can provide precise identification of a pathogen by using antibodies that are directed against a specific organism.

Culture

- Antibiotic therapy administered presampling can falsely render samples culture negative.
- Most human pathogens are fastidious, requiring media supplemented with nutrients to support growth and increase their numbers to detectable levels.
- Growth on solid media allows organisms to be separated into individual colonies; a pure (clonal) population permits subsequent identification and susceptibility testing.
- Selective agents such as antibiotics or dyes may be used to suppress unwanted organisms in specimens with a normal flora.
- An appropriate atmosphere must be provided: fastidious anaerobes require an oxygen-free atmosphere.
- Most pathogenic bacteria are incubated at 37 °C, but some fungi are incubated at 30 °C.

Identification

Bacterial identification depends on colonial morphology on agar, microscopic morphology, biochemical tests and, increasingly, nucleic acid amplification tests (NAATs) and gene sequencing. This is especially important for organisms that are slow growing (e.g. *Mycobacterium tuberculosis*) or impossible to grow (e.g. *Trophyrema whippelii*) to grow.

- Identification predicts pathogenicity: *Vibrio cholerae* causes severe watery diarrhoea, whereas *Shigella sonnei* infection is usually mild.
- Identification of some organisms should prompt public health action, for example contact tracing for a patient found to have meningococcal meningitis.

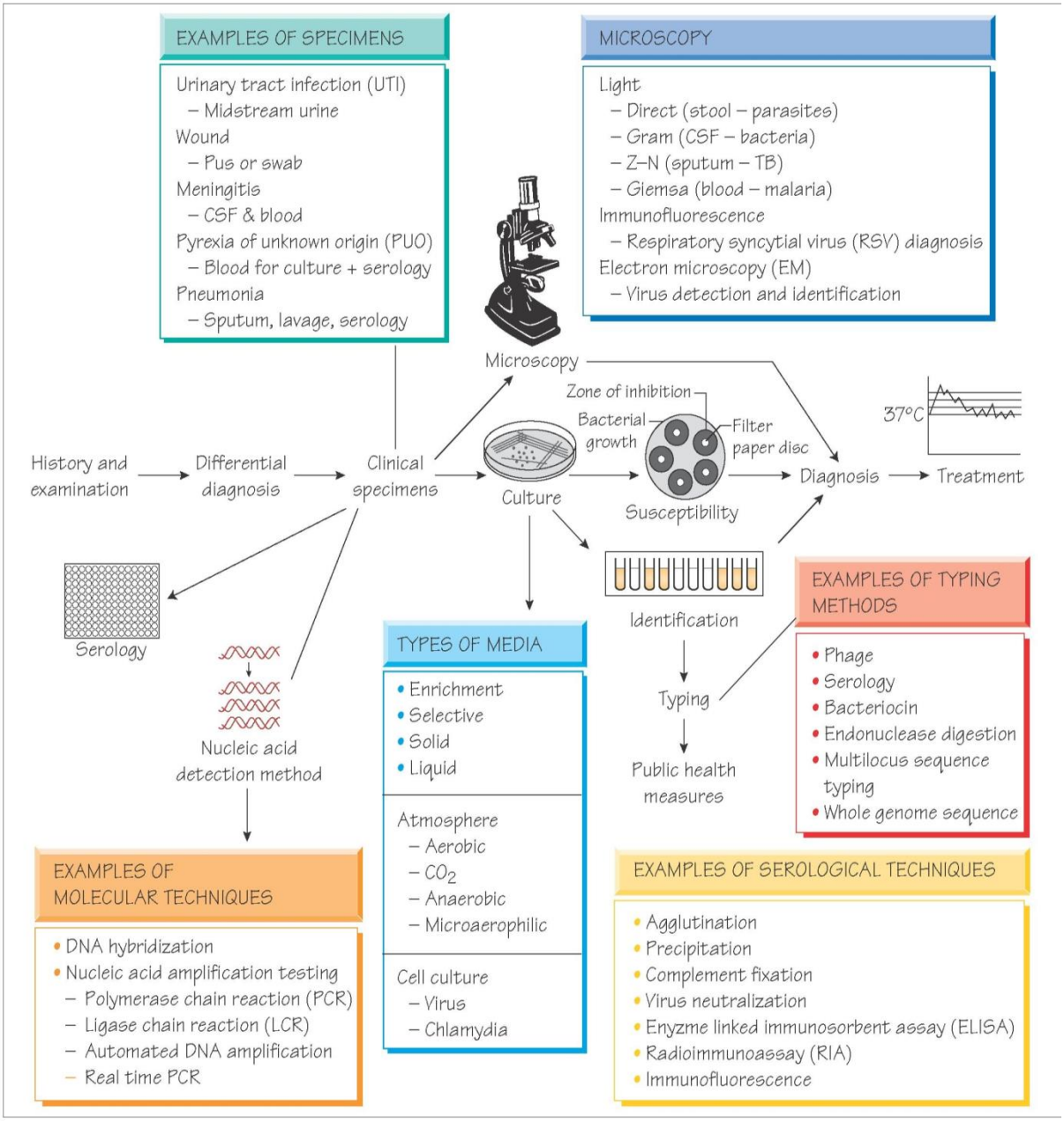
Susceptibility testing

Susceptibility testing aims to determine whether treatment with a given antibiotic will be successful. A susceptible organism should respond to a standard dose of an antimicrobial, a moderately resistant strain should respond to a larger dose, whereas a resistant organism is likely to fail therapy with the given antibiotic. Clinical response depends on host factors, and in vitro tests only provide an approximate guide to therapy.

Several bodies including the British Society of Antimicrobial Chemotherapy (BSAC) and the Clinical Laboratory Standards Institute (CLSI) define methods and standardized conditions to ensure testing is reproducible. Both are based on measurement of the diameter of the zone of inhibition of confluent growth for the test organism that is caused by an antimicrobial incorporated into a paper disc. The minimum inhibitory concentration, which is the lowest dose that completely inhibits growth, is a more objective method and enables resistance levels to be related to the concentration of antibiotic that is achievable in the tissues.

Susceptibility can be assessed rapidly by hybridization or sequence-based methods that detect specific antibiotic-resistance mutations.

THE LABORATORY INVESTIGATION OF INFECTION



Serology

An infection can be diagnosed by detecting the immune response to the pathogen: for example by detection of rising or falling antibody concentrations more than a week apart, or by the presence of a specific IgM or specific antigen. These techniques are used for organisms that are difficult to grow such as viruses (e.g. HIV or hepatitis B).

- Immunological techniques are used in diagnosis of infectious diseases. They are based on detection of:
    a) Presence of infectious agent (*antigen*, Ag)
    b) Detection of *antibodies* (Ab) – this means the patient has developed a specific immune response to the infectious agent.

- Reactions between Ag and Ab are highly specific, meaning the Ag will react in a highly selective matter with the corresponding Ab or sensitized lymphocytes, instead of just producing a large number of antibodies.
- The diagnosis of infection is usually based on demonstrating an increasing antibody levels (titer).
- The first serum should be collected during the acute phase of illness and the second serum should be taken ~2 weeks later.
- **IgM** always proves disease.
- **IgG** only proves disease in case of a 4x fold increase in titer!

Precipitation

- Reaction of soluble antigens like microbial toxins with antibodies, if there are enough complexes formed it will cause precipitation. There are 2 types of precipitations:
    - **Ring precipitation ---** is observed in a test tube. If concentration of Ag and Ab is optimal, then precipitation will appear.
    - **Precipitation in gel ---** gel diffusion test is used to demonstrate toxin production of *Corynebacterium diphteriae* (**ELEK test**).

Quellung test

- It is used for identification of *S. pneumoniae* – the capsule antigen will react with specific antibodies and this can be observed using a microscope.

Complement Fixation Test

- Complement is an enzymatic system of serum proteins that is activated by many types of Ab-Ag reactions (complexes). This activation is used to test for Ags or Abs. The test determines the presence of absence of Abs in the serum that is capable of fixing complement. Some Abs don't fix to complement, so the results of the test should be interpreted in terms of presence/absence of complement fixing Abs, rather than presence/absence Abs in general.
- A principle of competitive binding is important for understanding this test = labeled Ags compete with unlabelled Ags for the binding sites on specific Abs.

This stage can be visualized in 2 stages:

1) Test system:

- In this case the antigen is cardiolipin (lipid extract from beef).
- The patient's serum is heated at 56C for 30 min to inactivate the complement, then male guinea pig complement is added instead– the antigen and serum is mixed with the complement. If the patient has antibodies for the specific disease then Ag-Ab complexes will form and fix to the guinea pig complement.

2) Indicator system:

- A suspension of *sheep RBC* is added to the test system mixture to determine if unbound complement is available.
- The sheep RBC are sensitized with anti-sheep RBC called *Hemolysin* (causes destruction of RBC releasing hemoglobin).

Results:

- **Positive result** = the patient has Abs to the disease ⍰⍰the complement has been fixed in the first test (test system) therefore there is no hemolysis in the indicator system.
- **Negative results** = patient does not have Abs ⍰⍰meaning the complement has not been fixed (they are "free") – you will be able to see lysis in the indicator test.
- The test has allot of controls, to make sure the RBC don't lyse spontaneously in absence of complement and to make sure that the complement survives stage 1 in absence of Ag-Ab reaction.
- Complements are most effectively fixed in Ab excess or when Ag and Ab are in proper proportion to form maximum binding to each other. Ag excess tends to result in poor fixation of complement.
- Complement fixation is often used to test for **Syphilis** (*Treponema Pallidum*).

Agglutination

- Is similar to precipitation, except the *antigen* is not soluble, but *particulate*. There are 2 types:

1) Slide agglutination:

- For identification of different antigens of different bacteria:
  - *E. Coli* (K and O antigen)
  - *Salmonella* (O and H antigen)
  - *Shigella* (O antigen)
- These are all tested for epidemiological purposes. One drop of specific antiserum is put onto a slide and then bacterial colony is added – mixed well. If the test is positive you will the agglutination (lumps formed).
- Co-agglutination test: for identifying beta-hemolytic streptococci.

2) Tube agglutination:

- Makes a 2 fold dilution of the patient's serum (the specimen is the serum) - here the question is if the patient has specific antibodies or not.
- The particular antigen (bacterium) is added and incubated at 37°C – agglutination will be seen in some tubes. The titer (the highest dilution), is that dilution of serum in which you can still see agglutination (the concentration of serum in which agglutination is still possible).
- The tube agglutination test in some cases has special names, depending on the bacteria:

- *Salmonella typhi* ---- **Gruber Widal reaction**.
- *Rickettsia prowazeki* ---- **Weil Felix reaction**.
- *Brucella* sp ---- **Wright reaction**.

RIA (Radio Immuno Assay)

- Based on competition between *radioactively labeled* (known) and *unlabelled* (unknown) antigens for specific antibodies.
- Complexes forming between antigen and antibody can be separated in a radioactive way.
- RIA is very sensitive (nanograms/ml) method applied to the assay of *hormones* or *drugs* in serum – the concentration of specific Ag in the unknown sample ca be determines.

Disadvantage: radioactivity is dangerous.

Allergic Skin Test

- Based on Type IV (delayed type) Hypersensitivity.
- The bacterium itself or its proteins (e.g. PPD of *Mycobacterium*) are inoculated *intracellularly* and the reaction is checked 48-72 hours later.
- In positive reaction there will be erythema and edema.
- This test is performed for:

- *Leprosy*
- *Tuberculosis (Mantoux test)*
- *Brucellosis*
- *Tularemia*
- *Psittacosis*
- *Lymphogranuloma Venerum*

Toxic Skin Test

- Used to demonstrate sensitivity or protection against *exotoxins*.
- Diluted toxins are inoculated intracellularly. If patient has antibodies (*protected*) there will be no reaction (because the Abs *neutralizes* the toxin). However, if the patient is sensitive (no antibodies) it will result in a positive result --- erythema of skin.

This test is performed for:

- *Streptococcus Pyogenes* – **Dick test.**
- *Corynebacterium Diphteria* – **Shick test**.

Immunofluorescence

- Fluorescent dyes can be attached to antibodies and then observed by UV-light in fluorescent microscope. The labeled antibodies can be used to *identify antigens* on the surface of bacteria or in cells in histological specimens.

There are 2 ways of performing the test:

1) Direct :

- This is when *known labeled antibody* interacts with *unknown antigen* (indirectly).

2) Indirect:

- A known antigen is attached to a slide and the patient's serum with unknown serum antibodies is added. If the serum antibodies react with the antigen, they will remain fixed to the slide even after washing and can then be detected by adding a fluorescent-labeled antiglobulin (second antibody).
- This test is more sensitive than the direct test because more than one labeled antibody adheres per antigenic site.
- The method is widely used to rapid identification of bacteria and viruses.

Sandwich Technique

- Used to *identify antibodies in tissue.*
- Known antigen is added to the tissue and is then abound by a specific (unknown) antibody present in the tissue.
- Specific fluorescent-labeled antibody (known) is added which reacts with the fixed antigen.

Flow Cytometry (FAC – Fluorescent Activated Cell Sorter)

- It analyzes a single cell suspension flowing through a set of laser beams in order to measure the relative amount of light scattered by microscopic particles (giving info about relative size and granularity) and the relative fluorescent of the particles.
- For a mixture of white blood cells, it is easy to separate the cells into major classes e.g. small lymphocytes separated from granulocytes which are larger and contain more granules (scatter more light).
- This technique is used in clinical medicine and biochemical research.

ELISA (Enzyme Linked Immuno Sorbent Assay).

- In order to measure antibodies, known antigens are fixed to a solid phase (plastic microdilution plate), incubated with test serum dilution, washed and reincubated with anti-immunoglobulin labeled with an enzyme (e.g. horse radish peroxidase).
- Enzyme activity, measured by adding the specific substrate and measuring the *color reaction*, is a direct function of the amount of antibody bound.

Western Blot aka Immunoblotting.

- It is used to identify a particular antigen in a complex mix of proteins.
- The mix of proteins is subjected to sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. This separates the proteins according to molecular size. The proteins are transferred to a filter (microcellulose) so that the position of the elected proteins corresponds to their position in the gel. The filter reacts with a specific antibody, which binds only to epitopes of the proteins. The Ag-Ab complex can be detected by an enzymatic reaction or by radiolabeled reaction.
- Western Blot is useful for confirmation of HIV-1 virus seropositivity. (First always test with ELISA, if it is positive, then check again with Western Blot).

Molecular techniques

- DNA hybridization
- Nucleic acid amplification testing
  - Polymerase chain reaction (PCR)
  - Ligase chain reaction (LCR)
  - Automated DNA amplification
  - Real time PCR

Diagnosis of Viral infections

Virus diagnosis is important in case of:

- Life threatening infections
- Infections for which intervention exists
- Chronic infections
- For public health:
  - Keeping blood supply virus free (blood transfusions)
  - Monitoring and enforcing vaccination strategies
  - Identifying outbreaks
  - Recognizing presence of exotic viruses
  - Identifying new agents

Diagnosis can be done by:

1) **Direct examination**, where the clinical specimen is examined directly for the presence of virus particles, virus antigens, viral nucleic acids and virus-induced histological changes. The main advantage of direct examination methods is the short length of time required for a result, often within the same day.

Examples indirect examinations:

- Electron microscopy
- Light Microscope examination of specimen
- Immunofluorescence staining of specimen, with microscopic examination

❖ **Electron microscopy**

- Can resolve image down to ~200 nanometer, which is the size of the largest virus (filo-/poxvirus). Infected cells get prepared so that the electron dense material coats

  the structures that have to be visualized. Then an electron beam is passed through the samples while the electron magnet focuses the beam. The sample scatters the electron and results in an image.
- Can't usually differentiate virus species by visualization however, *virus families* tend to have characteristic appearances.
- Electron microscope is not usually found in standard medical laboratories.

2) **Indirect examination**, where the virus in the clinical specimen is amplified by growing in tissue culture, eggs or animals. Cell culture is by far the most commonly used method. The presence of growing virus may be revealed by changes such as cytopathic effect (CPE) or the ability to haemadsorb. The identity of the isolated virus can be further confirmed by various tests e.g. virus neutralization, immunofluorescence, complement-fixation, electron microscopy etc of virus in the clinical sample or by detecting presence of *antibodies* in virus serum.

Examples indirect examinations:

- Inoculation into animals
- Inoculation into embryonated eggs
- Isolation in cell culture

❖ **Cultivation**

It should be noted that viruses replicate only in living cells (bacteria-in free cell media) and also get inactivated in room temp. and therefore must be transported quickly.

- Viruses that have narrow host range are often grown in *primary cell cultures* (cultures taken *directly* from living individuals). However, these cells grow poorly and for limited times in culture. Diploid cells (fibroblasts) grow for 50-60 passages.
- Cells that grow well can be transferred indefinite number of times.
- Other viruses are grown in *cell lines* which are genetically changed from natural cells and are often gotten from tumors e.g. Hep-2 (laryngeal carcinoma).

Viruses will produce characteristic Cytopathic Effect (CPE), which can be:

- *Degeneration* _ gradual deterioration of cell w/ corresponding loss of function

- *Aggregation* _ breakup, divide. An example of aggregation s when the virus infected cells aggregate w/the proteins found on the surface of RBC, this is called ***Hemagglutination***.
- *Syncytium* _ a mass of cytoplasm with many nuclei but no internal cell boundary
- *Inclusion bodies* _ nuclear or cytoplasmic aggregate of stainable substance, usually proteins (capsid) e.g. Negri bodies (inclusion bodies seen in Rabies virus in neurons)

In case the virus doesn't produce CPE, its presence can be detected by:

1) **Hemadsorption:** when RBC attaches to surface of virus infected cells. This method is limited to viruses w/hemaglutinin (protein) in their envelope (so only in viruses can produce Hemagglutination), e.g. mumps, parainfluenza and influenza.
2) **Virus interference:** done by the formation of CPE w/ another virus. For instance rubella does not cause CPE, so it can be detected by placing it w/ certain enteroviruses which do cause CPE (echovirus, coxsackievirus).
3) **A decrease in acid production by infected/dying cells:** in this case a change in the color of phenol red (Ph indicator) would indicate virus infected cell and separate them from non infected cells. If the color remains red (alkaline) _ virus infected cell. If the color changes to yellow _ normal living cells (the change to yellow is due to acid production by the cells).

3) Serology

Serology remains the bulk of the work carried out by a routine diagnostic virus laboratory. A large variety of serological tests are available e.g. complement-fixation (CFT), hemagglutination-inhibition (HAI), enzyme-linked immunoassay (EIA), radioimmunoassay (RIA), particle agglutination, immunofluorescence, single radial haemolysis, western blot etc. The sensitivity and specificity varies greatly between different techniques. Most techniques will detect all classes of antibody whereas some assays e.g. RIA, EIA and IF can be made to detect one specific class only i.e. IgM, IgG or IgA.

❖ **Antigen detection**

Viral antigens can be found in patient's blood or body fluids using:

1. **ELISA:** test for the p24 antigen of HIC and surface antigen of Hep. B virus (most common examples).
2. **Latex agglutination:** antigens are agglutinated with special test sera (known antibodies) _ ***the specific binding of antigen with the antibody directly results in a reaction visible with the naked eyes.*** E.g. fast detection of rotavirus, adenovirus from stool sample.

4) Molecular Diagnosis

- Enzyme immunoassay (presence of antigen)
- Polymerase chain reaction, PCR (presence on nucleic acid)
- Southern/Northern hybridization (presence of nucleic acid)

Acute infection is diagnosed *by IgM. antibodies* or by a *4x increase in IgG titer* (titer = highest dilution of serum sample that gives positive reaction in the test).

❖ **Nucleic acid detection**

- Nucleic acid = viral genome/viral RNA.

- It can be detected in patient's blood or tissues with complementary DNA or RNA (cDNA or cRNA) as a probe. If there is little RNA in a patient's blood, like in HIV patients, then *Polymerase Chain Reaction (PCR)* can be used to amplify the specimen.
- In PCR »» take 2 small sections of single stranded DNA complementary to 2 regions of the target viral sequence; this is then reacted with the sample and an enzyme (DNA polymerase) that is capable of replicating DNA. The sample is then heated to release the newly made DNA strand. This process is repeated for ~ 20-40 cycles, each time using both original and newly made DNA as template for further replication to rapidly increase the population of a specific DNA. The large
- population of DNA is easily detected and techniques can be used to confirm that it is genetic info from a specific virus.
    - Special PCR reactions: quantitative competitive PCR, real time PCR and RT-PCR.

DIAGNOSTIC PROCEDURES IN FUNGAL INFECTIONS

1) Direct microscopic examination:

- Done with specimens like sputum, lung biopsy material and skin scrapings.
- Depends on finding characteristic asexual spores, hyphae or yeast in light microscope.
- The specimen is either treated with 10% KOH to dissolve tissue material leaving alkali-resistant fungi intact or stained with special fungal stains e.g. Indian ink, Calcofluor white (fluorescent dye), Methenamine silver stain.

2) Culture of the organism:

- Fungi are isolated on **Sabouraud agar** which contains glucose and beef extract, cicloheximid, antibiotics and blood.
- A low pH of 5.6 and two different temperatures are needed _ 25C (mold) and 37C (yeast).
- The medium facilitates growth of slow growing fungi by inhibiting the growth of bacteria with the low pH and added Chloramphenicol and Cloheximide e.g. to see spherules of C. immitis or capsule of C.neoformans.

3) DNA probe test:

- Can be used to identify colonies growing in culture at an early stage of growth, this way identification can be made more rapidly.
- Used for Coccidioides, Histoplasma, Blastomyces, Cryptococcus.

4) Serological tests:

- Identifying the presence of antibodies in patient's serum is useful in systemic mycoses, but less in other fungal infections. Like in bacterial and viral infections, one has to see a significant rise in antibody titer to confirm diagnosis.

  a) Complement fixation: test used frequently in suspicion of Coccidioides, Histoplasmosis and Blastomycosis.

  b) Latex agglutination: test of spinal fluid is used for detection of polysaccharide antigens of C.neoformans in case of Cryptococcal meningitis.

PATHOGENESIS OF FUNGI

- In response to infection many fungi form *granulomas* _ they are formed in the major systemic fungal infections and involve *cell-mediated immune response*. Seen in coccidioidomycosis, histoplasmosis and blastomycosis.

- Acute suppuration with presence of neutrophils n exudates occur in certain fungal diseases like aspergilliosis and sporotrichosis.
- Fungi do not have endotoxin in their cell wall and do not produce bacteria type exotoxins.
- Activation of cell mediated immune system results in a positive *delayed hypersensitivity skin test* in response to certain fungal antigens injected intradermally. A positive skin test shows exposure to fungal antigens – but does not imply current infection, because the infection might have occurred in the past.
- Intact skin is an effective host defense against certain fungi (e.g. Candida, Dermatophytes) – fatty acids in the skin inhibit dermatophyte growth and the hormonal changes in the skin during puberty limit ring worm of scalp caused by Trichophyton.
- The normal flora of skin and mucosa membrane suppresses fungi, when the normal flora is inhibited, like by antibiotics, overgrowth of C. Albicans can occur.
- In respiratory tracts, the important host defenses are the mucous membrane of nasopharynx _ traps inhaled fungal spores and alveolar macrophages.
- Circulating *IgG* and *IgM* are produced in response to fungal infection, but their role in protection from disease is uncertain.
- Cell mediated-response is protective, so suppressing it can lead to reactivation and dissemination of asymptomatic fungal infection and diseases caused by opportunistic fungi.

FUNGAL TOXINS AND ALLERGIES

- In additions to mycotic infections, there are two other types of fungal diseases:

1) Mycotoxicoses, caused by ingesting *toxins*:

a) Amanita mushrooms:

- Amanita toxin _ inhibits cellular RNA polymerase, which prevents mRNA synthesis.
- Phalloidin toxin

b) *Claviceps purpura* (mold):

- Infects grains and produces alkaloids
- Causes serious vascular and neurological effects

c) *Aspergillus flavus*:

- Produces alfatoxins (is a coumarin derivative)
- Causes liver damage and tumors in animals + suspected of causing hepatic carcinoma in humans.
- Alfatoxins are ingested with spoiled grains and peanuts and metabolized by the liver to the epoxide _ a potent carcinogen which induces a mutation of p53 tumor suppressor gene _ leading to loss of p53 protein and consequently loss of growth control in the hepatocytes.

2) Allergies

- Allergic reaction to fungal spores, especially to *Aspergillus*.
- Manifested primarily by an asthmatic reaction (rapid bronchochonstriction mediated by IgE), eosinophilia, and a “wheal and flate” skin test reaction.
- These clinical findings are caused by an immediate hypersensitivity response to the fungal spore.

Diagnosis of Parasitic disease

The effective control and treatment of parasitic diseases requires rapid, reliable and highly sensitive diagnostic tests, which can also serve to monitor the effectiveness of the therapeutic and prophylactic protocol.

Laboratory diagnosis is a basic step in the evaluation of the disease process,

1. **Direct identification**
2. **Indirect identification**
3. **Immunological diagnosis**
4. **Nucleic acid-based diagnostics**

1. Direct identification

Parasitic infections are usually diagnosed from samples of faeces, urine, blood and tissue.

I. Faeces:

Evidence of intestinal parasitism, apart from the general clinical signs, is obtained from faecal or post-mortem examination. In fact, a reliable diagnosis can usually be made only by using a combination of several techniques, such as:

1. Direct saline smear: This procedure provides only an indication of the parasites present and cannot be used quantitatively.

i. To prepare a direct faecal smear a drop of saline is placed in the centre of a microscope slide and a 2 mg faecal sample is suspended in this drop without spreading it.
ii. This is then covered with a coverslip and examined.

2. Stained smears: This type of smear is essential for accurate diagnostic detail and is also suitable for long-term storage for record purposes. The two stains generally used are haematoxylin and trichrome.

3. Parasite concentration in faeces by flotation: This is used for the identification of oocysts of coccidia and helminth eggs. One drawback of this technique is that there is not always a direct relationship between the number of eggs in faeces and the number of parasites present.

II. Urine:

Examination of urine sediment is used mainly for the identification of ***Encephalitozoon cuniculi*** and ***Schistosoma*** eggs.

III. Blood:

Testing is used to identify the various stages of blood parasites and is routinely applied to diagnose malaria, theileriosis, babesiosis, anaplasmosis, ehrlichiosis, trypanosomiasis and most types of filariasis. Trypanosoma can also be diagnosed with wet smears. Depending on the application and purpose, two types of blood films are used.

Thin blood films are useful for studying morphological changes of blood cells and blood parasites.

Thick blood films contain 6 to 20 times as much blood per unit area as thin films. The thick film is suited for rapid diagnosis of parasitaemia that is too low to be detected with thin films.

IV. Tissue:

Recovery of protozoa or helminths from biopsy material is often an important aid to diagnosis. Lymph node, spleen, liver, lung, bone marrow or spinal fluid biopsies are frequently used to diagnose a variety of diseases.

Concentration Methods

Concentration may be done using fresh or preserved feces. Several concentration techniques have been described.

They can be classified as

i. Floatation or
ii. Sedimentation methods.

In floatation method, the feces are suspended in a solution of high specific gravity so that parasitic eggs and cysts float up and get concentrated at the surface.

In sedimentation method, the feces is suspended in a solution with low specific gravity so that the eggs and cysts get sedimented at the bottom, either spontaneously or by centrifugation.

2. Indirect identification

Culture methods

Many parasites can now be grown in culture, but this has not become a routine diagnostic method in parasitic infections. It is sometimes employed for accurate identification of the parasite species. It is more often employed for obtaining large yields of the parasite as a source of antigen, for animal inoculation, drug sensitivity testing, for experimental or physiological studies and for teaching purposes. Some of the culture methods used for different parasites is indicated below.

1. *E. histolytica* and other intestinal amoebae can be grown in diphasic or monophasic media. **Boeck and Drbohlav's diphasic** medium and **Balamuth's monophasic liquid** medium is also used commonly for cultivation of amoebae and other intestinal protozoa. Amoebae can be demonstrated in the liquid phase in unstained mounts or stained smears.
2. **Novy and Macneal (NNM) medium** for cultivation of Leishmania is equally satisfactory for Trypanosomes also.
3. About 10-12 ml of defibrinated or heparinised blood rich in ring forms of malaria parasite, mixed with 0.2 ml of 50% dextrose solution using RPMI 1640 medium.

Animal inoculation

Animal inoculation can be used for isolating *Toxoplasma gondii* from infected persons. Lymph node or other biopsy materials are inoculated intraperitoneally into immunosuppressed mice. Peritoneal fluid obtained 7-10 days later may show the parasite in Giemsa-stained smears. However, serial passages may be necessary for its isolation.

Brain smears may be examined for cysts after sacrificing the mice 3-4 weeks after inoculation. Seroconversion of the animal also indicates a positive result.

Bone marrow, liver, spleen or lymph node aspirates from kala-azar patients injected intraperitoneally into hamsters is a very sensitive method. Even a single amastigote can establish the infection in the animal.

Spleen smears taken 4-6 weeks later show LD bodies. Blood from patients with trypanosomiasis can be injected intraperitoneally or into the tail vein of mice or rats. Parasitaemia can be demonstrated in 2 weeks.

3. Immunological diagnosis

Serology

Several serological tests have been developed for detection of antibodies to parasites using antigens from cultured parasites or from natural or experimental infections in animals or humans.

Virtually all types of serological reactions have been used. However, serodiagnosis in parasitic infections has only limited value due to various factors. Parasites are complex antigenically and exhibit wide ranging cross-reactions so that serological tests are not sufficiently specific.

In general, indirect haemagglutination (IHA), ELISA and counter immune electrophoresis (CIE) are most sensitive; indirect immunofluorescence (IF) and CF moderately sensitive; and simple precipitation in gel and coated particle agglutination least sensitive.

4. Nucleic acid-based diagnostics

The use of nucleic acid probes in the diagnosis of parasitic infections is based on the premise that every organism carries unique DNA sequences which differentiate it from other organisms. A diagnostic probe is developed by identifying and isolating these sequences. Sensitivity of detection depends on the abundance of unique sequences in the parasite genome. The most sensitive DNA probe is one originating from total genomic DNA since all the nucleic acid sequences of the organisms would be present in such a genomic probe. Genomic probes for ***Plasmodium falciparum*** can detect as little as 40 parasites per microlitre of blood. The specificity of nucleic acid probes is due to the ability of DNA to denature under certain conditions and to renature in a highly specific manner.

The specificity of a nucleic acid probe therefore depends on its base sequence. By using the Southern transfer technique it is possible to detect a single-copy gene with a probe of less than 20 nucleotides long.

1. **Hybridisation in the detection of parasite DNA**
   - **Detection of membrane-immobilised sequences**
   - **Detection of target sequences in solution**
2. **Labelling nucleic acid probes**
   - **Radioactive labelling**
   - **Non-radioactive labels**

With the large number of laboratories developing nucleic acid probes it is only a matter of time before solutions are found to the present probe-associated problems. Nucleic acid-based diagnostics will then become a matter of routine in the diagnosis of parasitic disease in the host and the parasite vector in epidemiological studies.