Medical Microbiology

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An Introduction to Microbiology

Microbiology traditionally is study of those organisms that are responsible for causing infectious diseases and their size makes them invisible to the naked eye. These include viruses, bacteria and related organisms. Other infectious agents which are larger and could be seen by the naked eye were called parasites and the study called parasitology.

This terminology and distinction between microbes and parasites is really arbitrary and not precise. All pathogenic microbes are essentially parasites and not some parasites could not be seen by the naked eye, besides all share some common features. So these days microbiology textbooks try to cover all infectious agents and tend to talk about microparasites and macroparasites rather than microbes and parasites.

Microparasites include *prions*, viruses, bacteria, protozoa and fungi. Microparasites are able to multiply within the host organism, although some protozoa also undergo some of their development outside the definitive host. Microparasites are so small that they are invisible to the naked eye. They are usually present in the host in such large quantities that epidemiologists usually count the number of infected hosts (prevalence of infection) rather than the number of parasites (intensity of infection). However, intensity can be measured for protozoans, which lie at the boundary of microparasites and macroparasites.

Macroparasites: include *helminths* and (the latter are usually). Macroparasites are not able to directly multiply within the host, and often need to leave the host to complete their life cycle. Macroparasites can usually be seen by the naked eye. This means that the number of parasites can be counted, and this has revealed that macroparasites are usually unevenly distributed within the host population. Often, many hosts are infected by a few parasites, and a few hosts are infected by many parasites. The effect on the host and the reproductive potential of the macroparasite often depends on the intensity of the infection.

Prokaryotes and eukaryotes

Bacterial cells are called prokaryotes and cells of all other organisms, except viruses, are eukaryotes. The main feature of a prokaryote is absence of a distinct nucleaue enclosed within a nuclear membrane. The DNA material is in form of a single circular chromosome floating in the cytoplasm. There are other DNA material in form of plasmids. In eukaryotic cells, the DNA material is in form of several chromosomes which is contained in the nucleus of the cell surrounded by a nuclear membrane. Cytoplasm of eukaryotes contains plenty organelles such as mitochondria, endoplasmic reticulum, Golgi apparatus and lysosomes, these are absent in prokaryotes.

Bacterial cell membrane is also covered by a thick cell wall which is absent in majority of eukaryotes. The cell wall in Gram positive bacteria is made of of peptidoglycan but in Gram negative bacteria there is an additional outer layer rich in lipo-polysaccharides.

Viruses, prions
Viruses are not true cells; they are mainly composed of genetic material(DNA or RNA) enclosed in a capsid or envelope without cytoplasm, cell membrane and the cellular machinery for protein synthesis. Prions, the agents which cause nvCreutzfield-Jacob disease and kuru appear to lack even nucleic acid and consist only of protein particles.

Living inside or outside the cells
Macroparasites and majority of microparasites live outside the host cells and take nutrients from the extra-cellular fluids. Viruses must live inside the cells because they rely on the host cell machinery for replication. They also get their nutrients from the intrcelluar fluids. Rickettsia and Chlamydia also live only within the cells.

Intracellular pathogens have the advantage of being largely protected from the body's defense mechanisms and effect of drugs. Extra-cellular pathogens on the other hand are under continuous action of body immunity but they have the advantage of ability to spread freely within the extra-cellular fluids and beyond.

Symbiosis
More complex forms of life and all animals are used as habitats by other organisms, even small animals and even protozoans have their own flora. The warm-blooded animals(birds and mammals) provide the best nutritious and favorable environment for the simpler forms of life and they are more widely colonized by those organisms. This form of living together where one organism lives inside or on the body of another organism is called symbiosis. Symbiosis can take 3 forms: commensalism, mutualism and parasitism.

Commensalism is the situation where one organism uses the body of a larger organism as its environment and may use this environment as a source of nutrition. For example normal flora of the mouth and skin. Commensal microorganisms are usually harmless but in certain conditions they may turn into pathogens.

When there is reciprocal benefits on the two organisms the relationship is called ***mutualism*** in which case the relationship is at least obligatory for one of them. It is not easy to draw a line between commensalism and mutualism. These organisms benefit the host by

- Preventing colonization of pathogenic species
- Producing certain substances that benefit the host such as flora of ruminant animals which produce metabolites that help digestion of cellulose.

In ***parasitism*** the symbiotic relationship benefits only on of the to organisms, the parasite. Classically parasitism means that the host is harmed by the parasite, which usually is the case, but not necessarily. Parasites may live within their host without causing disease.

Characteristics of parasitism
The following features are common to parasitism:

1. Many groups of organisms are parasitic including all viruses. All animals are parasitized.

2. Parasites take metabolic, nutritional and reproductive benefits from their hosts.
3. Some parasites are totally dependent and others partially. Viruses are totally dependent on their host cells for reproduction.
4. The disadvantage of parasitism to the parasite is that the development of the parasite is controlled by the host. If there is not suitable host, the parasite cannot reproduce and may die. That is why they have developed strategies for prolongation of survival such as cysts and spore formation.

The organisms

1) Prions

More primitive than viruses, protenaceous particles with little or no nucleic acid, very resistant to heat, replicate in lymphoid tissues and brain cells. Prions cause scrapie in sheep, bovine spongiform encephalopathy in cattle, nvJCD and Kuru in man.

2) Viruses

Viruses are composed of single or double strand nucleic acid(DNA or RNA) molecules contained in a capsule(capsid). This virus unit (virion) is called neuclocapsid. Some viruses are also surrounded by an outer envelope(enveloped viruses). Size varies considerably from very small (30 nanometer as poliovirus) to large (400 nm as vaccinea virus). Viruses are normally inert; they only become active and able to replicate when they enter a cell and use the cells machinery for replication.

Classification of viruses depends on type of nucleic acid(DNA, RNA), number of strands of nucleic acid (single strand or double strand), structure and size of virus particle(enveloped or non-enveloped), symmetry (icosahedral or complex) and mode of replication.

Viruses infect all forms of life and their ability to attack particular cells depends on their ability to attach to the host cell and get adsorbed by them. In some cases their are specific receptors on the cell wall to which the virus binds. When the virus enter the host cell cytoplasm, it sheds the capsid or the envelop and so the nucleic acid is released inot the cell. The virus is not infective at this stage until it replicates and new viruses are formed and expelled from through the host cell membrane.

Virus replication depends on host cell machinery but the virus must first synthesize its own mRNA. DNA viruses form mRNA by using host polymerase to transcribe mRNA from the viral DNA. RNA viruses use different mechanisms for mRNA synthesis which usually involves using their own enzymes (polymerase, reverse transcriptase) but sometimes the host polymerase too. The mRNA is then translated using the host cells mechanisms into viral proteins and nucleic acids. The new virus particles are then assembled into a new nucleocapsid. The enveloped viruses acquire their envelope from the host cell wall and some viral proteins duirng expulsion from the cell.

When the new generation of the viruses are released from the host cell, the host cell may undergo lysis and get destroyed as in case of infection with polio and influenza viruses (lytic infection). In case of other viruses such as hepatitis B, the infected host cell remains Alive and continues to release virus particles at a slower rate (persistent infection).

Major human pathogens include

- DNA viruses: herpes virus, varicella zoster, smallpox, hepatitis B, papilloma virus, adenovirus
- RNA viruses: polio virus, rota virus, reubella, HIV, hepatitis A, yellow fever, measles, mumps, rhinovirus, influenza, parainfluenza, rabies, Ebola.

3) The Bacteria

Huge numbers of free living non-pathogenic bacteria live in the nature and compared to these big numbers there are few species that cause human disease. Majority of pathogenic bacteria are known but from time to time new species are discovered.

Bacteria are prokaryotic cells lacking a proper nucleus and having a characteristic cellular organization. The cytoplasm contains many ribosomes but not other organelles found in eukaryotic cells. Many metabolic functions of eukaryotic organelles are performed by cell membrane. The genetic material of bacteria is composed of a double strand circular molecule of DNA which can be considered a chromosome with a continuous sequence of genes. The chromosome is tightly coiled into a region called necleoid but there is no distinct nucleus or nuclear membrane.

All bacterial cells except those of Mycoplasma are surrounded by a complex cell wall external to the cell membrane. The cell wall may be surrounded by a capsule, flagella and pili. The main structural component of the cell wall is peptidoglycan (mucopeptide). Bacteria are classified into Gram positive and Gram negative according to the type of the cell wall. In Gram positive bacteria the peptidoglycan forms a thick layer external to the cell membrane. In Gram negative bacteria the peptidoglycan layer is thin and is surrounded by another membrane formed mainly of lipopolysaccharides and acnchored to the peptidoglycan layer. The gram positive cell wall is hydrophilic and the gram negative cell wall is both hydrophilic and hydrophobic because of the lipid content. In Mycobacteria, the lipopolysaccharide layer turns into a think waxy layer which changes the gram stain property of the bacteria to acid-fast and makes the bacteria more resistant to drying and environmental factors.

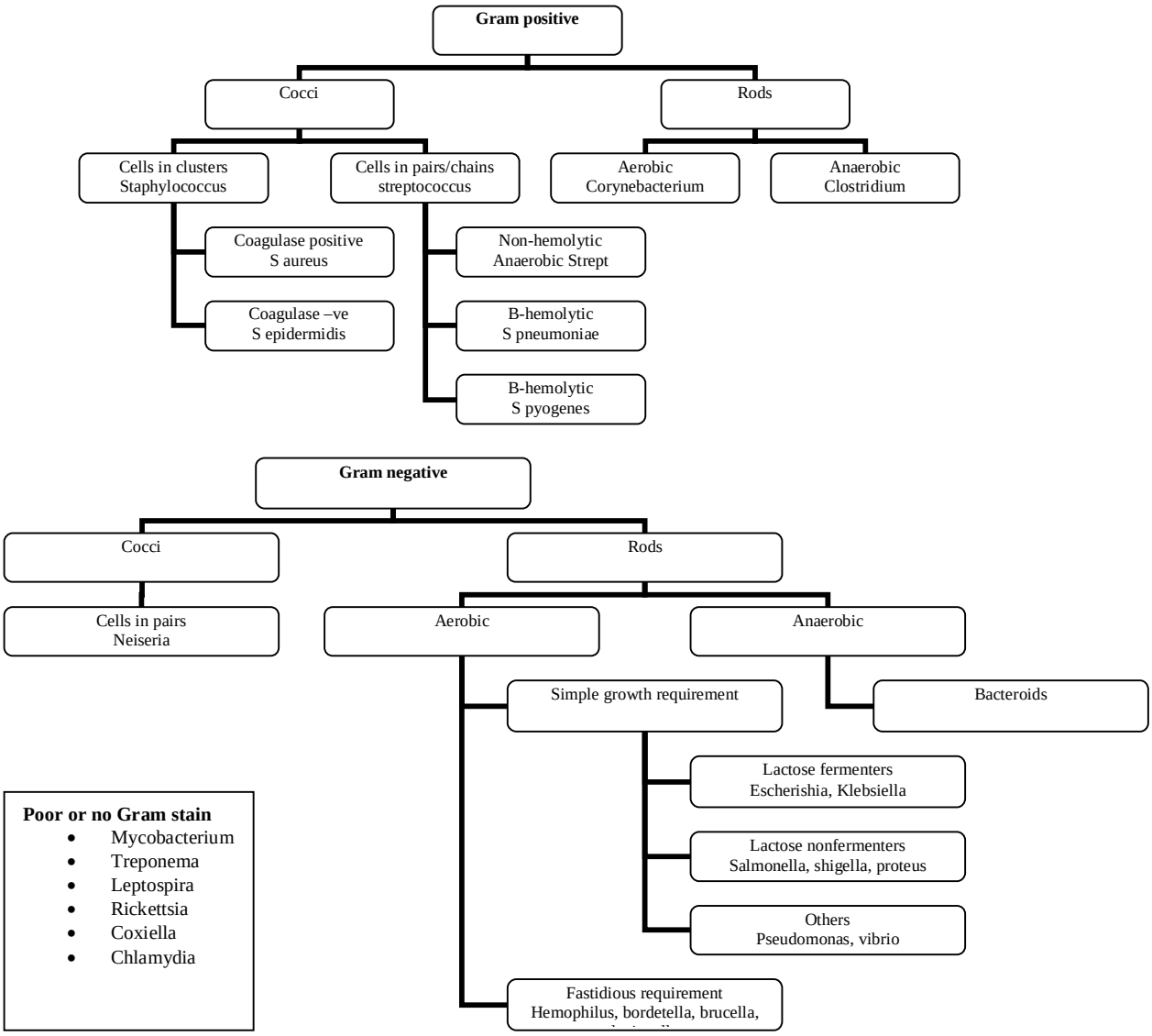
In some bacteria there is a capsule outer to the cell wall composed of polysaccharides giving a slimy surface and providing protection against host phagocytosis and thus is important in virulence.

Bacteria obtain nutrients by taking up small molecules such as amino acids, sugars and small peptides from the environment across the cell wall and transport of the molecules into the cytoplasm is achieved by the plasma membrane. All bacteria obtain energy in form adenosine triphosphate by oxidizing preformed organic molecules (carbohydrates, lipids and proteins) from the environment. Bacteria can metabolize these molecules with or without oxygen. Aerobic metabolism yields more energy that anaerobic metabolism. For example aerobic metabolism of glucose yields 38 molecules of ATP while anaerobic metabolism yields only 2 molecules of ATP. Therefore anaerobic respiration(fermentation) is less efficient but it has its advantage in ready availability of energy sources in the host's body. Some bacteria do always require oxygen for respiration(obligate aerobes), others can do with and without oxygen too (facultative aerobes).

Reproduction of bacteria is by simple division into two identical daughter cells. This can take 20-30 minutes and in E. coli or up to 24 hours as in M. tuberculosis. Before it can divide, the bacterial cell must duplicate its genetic material. The DNA replication involves unwinding of the two strands each of which then serve as a template for DNA polymerase. Each new genome is thus composed of one parental DNA strand and a newly synthesized one. After replication of genome a septum is formed in the middle of the cell and the cell then divides at the septum to form two daughter cells.

For improved survival under adverse conditions, some bacteria can form highly resistant endospores (within their cells). These spores are formed when the cell is unable to grow e.g. nutrients are exhausted but never by actively growing cells. The spores are extremely resistant to heat and chemicals, they can remain viable in a dormant state for many years recovering rapidly to normal existence when conditions improve. Spores of Clostridium and Bacillus are abundant in soil and of particular hazard (tetanus, anthrax).

Identification of bacteria of medical importance is made on the basis of the following characteristics: Gram stain, cell morphology (rod, coccus, pair or chains), ability to grow under aerobic and anaerobic conditions and growth requirements. Major groups of bacteria are shown in the figure.



4) The Fungi

Fungi are eukaryotes but they are distinct from animals and plants; characteristically they are multinuclear or multicelluar organisms with a thick cell wall growing as thread-like filaments called hyphae. The yeast forms grow as ovoid single cells. Some show both forms (yeast and filament) during their life cycle(dimorphic). Most familiar forms of fungi are mushrooms and single celled yeasts. They are used in baking and drug industry. Some species are pathogens.

The filamentous forms such as Trichophyton reproduce by forming sporangia from the hyphae which liberate spores and spread the fungus. These spores are a common source of infection by inhalation. The yeast forms such as Cryptococcus reproduce by division and budding. Dimorphic forms such as Histoplasma occur as yeasts inside the body but form hyphae in environmental temperatures. Candida is an exception of the dimorphic which form hyphae within the body.

Many fungi are pathogens and can cause disease(mycosis):

- Superficial mycosis where the fungus grows at the body surface and cause disease in skin, hair and nails. These are usually simple infections and spread by direct contact.
- Deep mycosis with involvement of internal organs. These are usually due to opportunistic growth of fungi in individuals with impaired immunity.
- Poisoning: Free-living fungi can cause disease in humans by producing toxins in edible items (aflatoxins).

Many fungi that cause disease are usually free-living organisms or normal flora of the body e.g. candida which do not cause harm unless the body's immunity is compromised. The major groups of pathogenic fungi are shown below:

Type	Anatomic location	Organism	Disease
Superficial	Hair, shaft, skin Epidermis, hair, nails	Malassezia Microsporum, trichophyton	Tinea versicolor Ringworm (dermatophytosis)
Deep systemic	Internal organs	Coccidoids Histoplasma Blastomyces	Coccidomycosis Histoplasmosis Blastomycosis
Opportunistic	Internal organs	Cryptococcus Candida Aspargellus	Cryptococcosis Candidiasis Aspargellosis

4) The Protozoa

Protozoa are unicellular animals many species of which can cause human disease especially in the tropical and sub-tropical countries. Some parasitic protozoa live inside the host cells and others live outside the cells in the blood, intestine and urogenital tract. Intracellular species obtain their nutrients from the host cell directly by up taking molecules and cytoplasm. Extracellualr species take nutrients from the extracellualr fluid or by ingesting host cells.

The grown stage of the protozoa is called trophozoites. Reproduction is asexual and usually happens in human body by binary or multiple division of the trophozoite. Sexual reproduction is normally absent or restricted to the insect vector phase. Cryptosporidium is exceptional in that it has got sexual reproduction in human body. Asexual division give

the advantage of rapid increase in the number of parasites in the host body which can be dangerous in immunocompromised hosts.

To avoid host immunity protozoa have evolved certain strategies to increase their chances of survival mainly variation in their antigens, the fact that makes it difficult to develop effective vaccines against them. For example Trypanosomes change their surface antigens repeatedly; malaria show polymorphism in the surface antigens; and amoebae can consume complements on the cell surface. Intracellular protozoa have evolved mechanisms to avoid the effect of host cell enzymes. Intra cellular protozoa may present their antigens on the host cell surface making them a target for body defense action.

Major medically important protozoa are shown in the following table:

Location	Species	Mode of transmission	Disease
Intestinal tract	Entamoeba histolytica Giardia lamblia Cryptosporidium	Ingestion of cyst	Amoebiasis Giardisais Cryptosporidiosis
Urinary tract	Trichomonas vaginalis	Sexual	Trichomoniasis
Blood and tissue	Trypanosoma cruzi Trypanosoma gambiense T. rhodesiense	Reduviid bug Tsetse fly	Trypanosomiasis Chagas disease Sleeping sickness
	Leschmania donovani L. tropica	Sand fly Sand fly	Visceral leismaniasis (kala azar) Cutaneous leishmaniasis
	Plasmodium species	Anopheles mosquito	Malaria
	Toxoplasma gondid	Ingestion of cyst in row meat Contact with soil contaminated with cat feces	Toxoplasmosis
	Pneumocystis carinii	Inhalation	Pneumonia

5) The Helminths

There are three main groups of parasitic worms that infect humans: the tapeworms (Cestoda); the flukes(Trematoda) and the round worms (nematoda).

The helminthes are larger organisms with variable sizes. The larval stages are usually very small but adult worms may be centimeters or even meter long. Infection is commonest in the tropical and sub-tropical countries, among children and in lower socioeconomic populations. Transmission can occur in 4 ways:

- Feco-oral by ingestion of infective eggs or larvae
- Ingestion of infective larvae in tissues of another host
- Penetration of intact skin by the larvae
- Bite of insect

Many worms live in the intestine while other live in deeper tissues. Almost all organs of the body can be parasitized. Flukes and nematodes actively feed on host tissues and intestinal contents but tapeworms absorb already digested nutrients as they lack a digestive system. Nematodes have separate sexes, most flukes are hermophrodites except schstosomes. Larvae of flukes and tapeworms must pass thru an intermediate host before

being able to develop to an adult, but nematodes develop into adult without intermediate hosts.

Tapeworms frequently infect men through eating undercooked or row meat containing larvae. Flukes can also infect humans and develop in liver, lungs, blood and intestine, the most notable one being schistosomes. Several species of Nematodes can cause infection in man. See table below for details.

Species	Transmission	Site of infection in man
Tape worms		
Taenia saginata T. solium Diphyllobothrium latum Hymenolepsis nana	Larvae in beef Larvae in pork Larvae in fish Eggs, larvae in beetles	Intestine
Larva of T solium	Eggs in food or water	Brain, eyes
Echinococcus granulosus (haydatid)	Eggs passed by dogs	Liver, lung, brain
Flukes		
Schistosoma species	Penetration of skin by larva	Blood vessels of bladder and intestine
Fasciola hepatica	Ingestion of vegetation with larva	Liver
Paragonimus westermani	Ingestion of crab with larva	Lungs
Nematodes		
Ascaris lumbricoides	Ingestion of eggs	Small intestine
Enterobius vermicularis	Ingestion of eggs	Small intestine
Hookworms(ancylostoma)	Skin penetration by larva	Small intestine
Strogyloides stercoralis	Skin penetration by larva	Small intestine
Trichuris trichiura	Ingestion of eggs	Large intestineq
Onchocerca volvulus	Bite of Simulium fly	Skin, eye
Wuchereria bancrofti	Bite of mosquito	Lymphatics and blood vessels
Loa loa	Bite of deer fly	Tissues
Toxocara canis	Ingestion of eggs passed by dogs	Tissues, CNS

6) Arthropods

Many insects feed on human blood including mosquitoes, midges, flies, bugs, fleas, ticks, lice and mite. These parasites live on the body surface(ectoparasites) except the scabies mite, Sarcoptes scabei which burrows into the superficial layers of the skin. Some arthropods transmit pathogens of all major groups such as viruses, bacteria and protozoa.

Pathogens	Disease	Arthropod vector
Viruses Arboviruses	Dengue fever Yellow fever Encephalitis Hemorrhagic fever	Mosquitoes Ticks
Bacteria Yeserina pestis	 Plague	 Fleas
Rickettsias	Typhus Spotted fever	Lice, fleas, ticks
Protozoa Trepanosoma species Plasmodium	 Tripanosomiasis Malaria	 Bugs, tsetse flies Anopheles mosquito
Worms Onchocerca	 Onchocerciasis	 Simulium flies

The normal flora

A large number of microorganisms, mainly bacteria, live on or in our bodies intimately without normally causing us harm. These indigenous microorganisms are called the normal flora. The normal flora are highly adapted to live and stay in their preferred areas. These microorganisms are also called the resident flora. There are also transient flora which are microorganisms from the environment that may be present in the host for hours, days or weeks and then disappear. The transient flora are usually not significant but in circumstances when the normal flora diminish the transient flora may colonize and cause disease.

The normal flora occur in those parts of the body that are exposed to or communicates with the external environment such as skin, nose, mouth, conjunctiva, the upper respiratory tract, the digestive tract and urogenital tract.. Certain parts of human body remains sterile including blood, the cerebrospinal fluid, urinary bladder, uterus, paranasal sinuses, the middle ear and kidneys.

The association between the flora nd the human host is poorly understood, but it is thought that there is a dynamic interaction between the two rather than association of mutual indifference. It is thought that the flora and the human body derive some benefit form the other and the relationship is mutualistic. The normal flora derive form the host supply of nutrients, a stable environment, constant temperature, protection and transport. The host can obtain some nutritional benefit from the normal flora a swell as stimulation of the lymphatic tissue, but the most important benefit is that colonization by the well adapted normal flora excludes other microorganisms from colonizing the site.

Origin of the normal flora

Before birth the healthy human fetus is sterile i.e. free of microorganisms. After normal vaginal delivery the newborn infant first encounters microorganisms while passing down the birth canal especially from the vagina. The fetus acquirs these microorganisms by surface contact, swallowing and inhalation. After this other microorganisms join the newborn's flora from the immediate environment including skin and the respiratory tract of people caring for him. Soon after exposure to full range of microorganisms from the surroundings, those microbes that compete best in particular sites remain and become predominant. These microorganisms form the stable flora. In a short time the child has the same kind of normal flora as an adult in the same environment.

In any given time, the flora represent reflect age, nutrition, hygienic practices and the environment of the individual.

It has been estimated that a human body consists of 10^13 and something like 10^14 bacteria associate to it in form of normal flora. Other organisms such viruses, fungi and protozoa are found in healthy individuals but these groups a small proportion of the flora.

Composition of the normal flora

The types of microorganisms that constitute the normal flora in corresponding sites in different animal species vary widely and are related to factors such as age, sex, diet and temperature. Some bacteria are constantly found in certain anatomical sites, others are present only occasionally or at certain times during life. However the composition of the

normal flora is sufficiently constant for a particular animal species. The lowest density of flora are found in the stomach and the highest density in the large bowel.

Normal flora of the skin

The skin is a hostile environment for bacteria because of its low acidity and presence of inhibitory substances such as fatty acids. But the normal flora can resist these factors. The density and composition of the normal flora of the skin vary according to the anatomical locality, being more in moist places. The axilla, groin, scalp and areas between the toes support growth of high numbers of bacteria. The density in other areas of the skin is very low in hundreds to thousands per square centimeter. The flora near body orifices are more abundant and resemble those in the orifice.

The majority of skin flora are found in the most superficial layers of the epidermis and the upper parts of the hair follicles. The commonest species is Staphilococus epidermidis accounting for 90% of skin flora. Staphylococcus aureus may be present in the moist areas especially in the nasal carriers. Anaerobic diphtheroids occur below the skin surface in the hair follicles, sweat and sebaceous glands. Candida may be present on the scalp.

Normal flora of the oral cavity and nose

The presence of nutrients, epithelial debris and secretions makes the oral cavity a favorable place for a large number of bacteria. Common bacteria colonizing mouth include streptococci, staphyilococci, diptheroids and anaerobes. The oral environment changes with age and with this the flora changes too. The oral cavity is sterile at birth but soon becomes colonized with the first feeding from maternal flora. Streptococcus salivarius is the dominant flora of mouth until tooth eruption accounting for more than 90% of the oral flora. With appearance of the teeth other species of streptococcus colonize such as S mutans and S sanguis.

Large numbers of anaerobes habit the surface of the teeth and gums. Plaques is a film of bacterial cells anchored to the polysaccharide matrix which they secrete. If the teeth are not cleaned regularly, the plaque may accumulate rapidly and the activities of S mutans may lead to dental caries as acid is fermented from carbohydrates and attacks the enamel.

The normal flora of the mouth can harm the host because dome bacteria has pathogenic potential. For example oral bacteria gaining access to other tissues through wounds may cause disease such as abscess of the alveolar bone, lung, brain and extremities. Porphyromonas gingivalis plays and important role in this pathology. Oral streptococci can also be introduced to the blood stream from dental manipulation and may lead to endocarditis in persons with heart valve problems.

Normal flora of the respiratory tract

The pharynx and trachea have their normal flora which may include hemolytic streptococci, staphylococci, anaerobes and Neisseria. But the lower respiratory tract i.e. bronchi and pulmonary tissues, are normally free from microorganisms despite regular intake of organisms with inspiration. This is mainly due to the effective cleansing effect of the ciliated epithelium that line the respiratory tract sweeping upwards any bacteria reaching the lower areas tract. These are them removed by coughing, sneezing or

swallowing. If the airway epithelium is damaged such as by chronic bronchitis or viral pneumonia, the individual may become susceptible to infection by pathogens descending from the nasopharynx.

The normal flora of conjunctiva

A variety of microorganisms can be cultivated on the conjunctiva but their numbers are small. More commonly seen organisms included Staphylococcus epidermidis and coryneforms. S. aureus, streptococci and Neisseria are occasionally found. The action of blinking every few seconds washes away foreign bodies on the conjunctiva including bacteria. Tears also contain bactericidal substances such as lysozyme which attack bacteria. Because of these defensive actions, bacteria(e.g. Chlamydia trachomatis and Neisseria gonorrheae) need special mechanisms to be able to infect the conjunctiva; they need to attach to the conjunctival epithelium and resist the action of lysozyme.

Nor mal flora of the urogenital tract

Urine is usually sterile and the urinary tract is continuously flushed by urine making it difficult for the microorganisms to reside and ascend through it. The anterior urethra has got a little flora including mainly Staph. epidermidis, Strept fecalis. Additionally some enteric bacteria such as E coli probably contaminated from the skin, vulva and rectum, are occasionally found at the anterior urethra.

The vagian becomes colonized soon after birth with corynebacteria, staphylococci streptococci, E coli and lactic acid bacillus (Doderlein's bacillus). From puberty until menopause, the vaginal epithelium contains glycogen. Dodelein's bacillus metabolizes glycogen to lactic acid which leads to a low PH of the vaginal epithelium. This acidity inhibits colonization of other bacteria and Candida albicans. Doerlein's bacillus thus becomes dominant on the vaginal epithelium.

Nor mal flora of the alimentary tract

The gastrointestinal tract contains huge numbers of different microorganism. The number and species different according to age, diet, cultural conditions as well as the position in the gastrointestinal tract.

The entire intestinal tract is sterile at birth. Bacteria enter with first feeding an colonization depends on the source of food. In breast-fed infants bifidobacteria account for more than 90% of the intestinal flora. Other bacteria are also present such as enterobactericeae and fecal streptococci. When breast-fed infants switch to other forms of food, the bifidobacteria are progressively replaced by enteric bacteria, bacteroids, streptococci, lactobacilli and clostridia. In bottle-fed children bifidobacteria are not predominant and the flora is usually not consistent.

The esophagus contains bacteria swallowed with saliva nd food. The stomach is not a good environment for bacteria growth because of the high acidity where only few bacteria mainly lactobacilli can be cultured.

The small intestine contains more bacteria. The proximal part mainly contains Gram positive flora consisting of lactobacilli and strept fecalis (10^5-10^7 bacteria per ml). the

distant part of small intestine contains a greater number of bacteria and additional species such as coliforms and bacteroids.

In the large intestine, the numbers become much higher (10^11 per mil) and the bacteria are more variable. Coliforms are more abundant and the prominent species are anaerobes (baceroids and bifidobacteria). These anaerobes may outnumber the E. coli by more than 1000 times. It is estimated that up to 400 species are usually present in the large bowel.

Different species have affinity to different parts of the GIT. Some bacteria occur in the lumen while other attach to the epithelium. Gram positive and Gram negative bacteria use different mechanisms to attach to the GIT epithelium using specific receptors I the epithelial cells.

Advantages and disadvantages of normal flora

The importance of the normal flora to the host sometimes becomes clear with broad-spectrum antibiotic therapy which can drastically reduce the number of normal flora and give chance for overgrowth of resistant species. For example clindamycin treatment can reduce enteric flora and lead to overgrowth of Clostridium difficile which can cause pseudomembranoues colitis. The advantages of flora include:

1. Preventing colonization of pathogens: normal flora can prevent colonization of other pathogens in several ways:
   - The huge number of the normal flora means that all ecologic niches are occupied and the flora outcompete the pathogenic species.
   - Skin bacteria produce fatty acids which can prevent growth and attachment of other species
   - Gut bacteria release certain antimicrobial factors (e.g. bacteriocins, colicins) which prevent establishment of other species.
   - Vaginal lactobacilli maintain an acid environment which suppress other organisms.

2. Gut bacteria release organic acids which may have some metabolic value to the host. They also produce viramin K and B vitamins in amounts large enough to be useful to the host.

3. The continuous interaction of the flora and the mucosa stimulates the development of the immune system.

The normal flora can also harm the host in several ways. They compete for food; they can cause infection if the host immunity fails and they can sometimes be displaced from their normal positions and cause infection elsewhere in the host. The normal flora can casue infection in the following situations:

1. If the intestine is perforated or skin is injured.
2. During extraction of teeth when strept viridans can enter the blood stream.
3. E. coli can ascend from the skin to the urinary tract and cause UTI.
4. When the immune system fails e.g. immunosuppresssion and HIV/AIDS.
5. When the composition of the flora changes and pathogenic members outgrow e.g. with antibiotic therapy.
6. When the local environment changes e.g. increase in stomach or vaginal PH.

Defense against infection

Innate and acquired immunity

Immunity is the ability of the organism to resist infection. The response that the body makes to against an infectious agent is called the immune response. This response is made by the immune system; the tissues, cells and molecules involved in defense mechanisms against infection.

When a microorganism enters the body for the first time, the defense system inherently present may be sufficient to prevent replication of the pathogen and thus prevent development of disease. These established immune mechanisms are called the innate immunity. However even if the innate system was unable to curb the infection, a set of other specific mechanisms gradually develop to control the infection. Thise specific defense mechanism is called the acquired or adaptive immune system which in the end eliminates the pathogen form the body.

The main feature of the acquired immunity is its specific memory to the pathogen against which it has developed so that in the next encounter, it promptly recognized the pathogen and attacks it. Innate immunity on the other hand, is not specific and has no memory for the specific pathogen. Both the acquired and innate immunity work in synergy and complement the body's fight against infection. The differences between the two systems are shown in the table below.

	Innate immunity	**Acquired immunity**
Origin	Present at birth	Develops with first encounter with pathogen/products
Specificity	Effective against all pathogens	Effective against a specific pathogen
Cells	Phagocytes, natural killer cells	T-lymphocytes
Soluble factors	Lysozyme, compliment, C-reactive protein, interferon	Antibody
Strength at first contact	+	+
Strength at subsequent contact	Same	Much stronger
Memory	No specific memory to the pathogen	Specific memory present
Repeated contact	Doesn't improve resistance	Improves resistance

The Innate immunity

Defense against entry of the pathogen into the body

Before being able to establish itself in the body, the pathogens must overcome a number of chemical and physical barriers at te body surfaces. The most notable ones are the skin and the mucous membranes.

The skin is normally impermeable for the majority of pathogens. Intact skin does not alow bacteria in. the low PH of the skin is hostile to bacteria. The lactic acid and the fatt acids present in the sweat and sebaceous glands inhibit growth of many bacteria. The mucous membranes lining the interior of mouth, respiratory tract, genitourinary tract and other hollow structures, secrete mucus which is a protective barrier. Mucus prevents adherence of bacteria to the epithelial cells and thus baring them from gaining access into the cells.

There is also a mechanical action by the ciliated epithelium, coughing and sneezing to wash away the bacteria from the body. In addition to this the body fluids contain a variety of inhibitory substances that work against the pathogens such as lysozyem in tears, nasal secretions and saliva; gastric acid in the stomach, lactoperoxidase in milk, and spermine and zinc in semen.

Apart from the body's own defense mechanisms at the body surface, there are the normal flora which contribute to the fight against establishment of pathogens on the skin and mucus membranes.

Defense once the pathogen has entered the body

Despite the presence of surface barriers, some organisms may be able to pass the surface and gain access into the body. When this happens two defense strategies are called into action:

- Phagocytosis: engulfment and killing of pathogens by specialized cells
- Destruction of pathogens by soluble bactericidal factors.

Phagocytosis

Professional phagocytosis of invading microorganisms is carried out by two families of cells:

- The large macrophages
- The polymorphs (neutrophils) which are smaller polymorphonuclear granulocytes.

The macrophages originate in the bone marrow from promonocytes which develop into circulating monocytes in the blood and finally mature to macrophages. Macrophages are founds widely in all tissues and associated with the basement membrane of the small blood vessels. They are especially numerous in the lungs(alveolar macrophages), liver(the Kupfer cells), lymph nodes and splenic sinusoids where they are well placed to filter out foreign material. Other examples of macrophages are brain microglia, kidney mesangial cells, synozial A cells and bone osteoclasts.

The neutrophils are the most abundant of white cell types in the blood. Like phagocytes, thesy also originate in the bone marrow. The charachteristic of neutrophc is its cytoplasm which contains a variety of granules which have antibacterial activity.

Phagocytosis of microorganisms involves several processes. The first event is attachment of the pathogen to the surface of the cell via nonspecific receptors. This attachment initiates a process of phagosome forming in which pseudopodes are formed around the foreign body to uptake it. These cytoplasmic arms form a vacuole around the organism and the cytoplasmic granules discharge their microbicidal contents into the vacuole aand ultimately kill the pathogen. .

Phagocytosis will not happen unless the microorganism is attached to the surface of the leukocyte and this will not happen unless the 2 are in close proximity. So there must be a mechanism which stimulates the migration of the leukocytes towards the pathogen. Many bacteria produce substances which directionally attract the white blood cells, a process called chemotaxis. However, this chemotaxis is a weak signaling system. A stronger defensive action is initiated by activation of the complement system.

Stimulated by presence of certain substances on the surface of bacteria, the so-called alternative complement pathway is activated which includes a cascade of complement processes leading to production of chemotactic factors and vascular permeability. The vascular permeability mediators increase permeability of the capillaries allowing exudation of plasma, fluid and more complement to the site of infection. These factors also induce margination (sticking) of the polymorphs to the wall of the capillaries. .

The chemotactic factors provide a chemical gradient for the attraction of marginated polymorphs from inside the capillaries through the wall to the extravascular environment and finally to the site of the bacteria coated with C3b complement which has initiated the whole process. The polymorphs have special receptors to bind to the C3b component which is now attached to the bacterial cells.

The process of capillary dilatation (erythema), exudation of fluid and plasma proteins(edema) and the accumulation of neutrophils is called acute inflammatory process which provide an effective way of aggregating the phagocytes in the site of infection and directing them to the complement-coated bacterial cells.

Certain plasma proteins such as C-reactive protein, called acute phase proteins, are increased during this acute inflammatory phase by stimulation from alarm mediators such as interleukins which are released during infection. Some of these protein have a role in the complement process and others have antibacterial activity.

There are many antimicrobial agents which operate at short range inside the phagocytes and in the body fluids such as lysozyme and lactoferrin. Cells infected with viruses synthesize interferon which has antiviral activity.

Natural killer cells attach to the surface of cells which are infected with viruses. This process of recognition leads to targeting of the infected cells which ends with apoptosis(programmed cells death) of the infected cells.

Phagocytosis is ofcourse not effective against large parasites such as helminthes. These parasites are targeted by Eosinopils. Many helminthes can activate the alternative complement pathway leading in the end to their coating with C3b. this coating will be targeted with Eosinophils which attach to the parasite and release their intracellular proteins to attack and destroy the pathogen.

The acquired immunity

The innate immunity is the first line of the body's defense against infection. This line often succeeds but sometimes fails as certain microorganisms manage to find ways around it. In these situations the adaptive immunity comes into action. In the acquired immune system response, when pathogens enter the body the lymphocytes lead an immune response depending on production of numerous antibodies specific to that pathogen and the magnitude of the response increases with time.

The role of antibodies : Defense against extra cellular pathogens

Antibodies are synthesized by B-lymphocytes which mature in the bone marrow when the lymphocyete come in contact with an infectious agent acting as a foreign antigen. Each antibody has got a recognition site specific to the antigen that induced its production and to

which it binds. When the antibody molecules bind an antigen, the resulting complex activates the classical complement pathway. In this way the pathogens are brought to the battlefield of the innate immune system during the acute inflammatory process.

Some antibodies (IgE) has specific affinity to receptors on the surface of Mast cells. When microbial antigens bind to these antibody-bound cells, the cell releases mediators for chemotaxis and vascular permeability.

Other sites of some antibody molecules have affinity to bind to receptors on the surface of phagocytes leading to phagocytosis of the pathogens.

Some antibodies bind to certain reacting molecules of the pathogen and block its function. For example and antibody directed against the influenza agglutinin will prevent the virus form attaching to to its preferred receptors on the host cells.

Structure of antibodies

Antibodies are immunoglobulin molecules consisting of two identical light polypeptide chains and two identical heavy polypeptide chains. These chains are bound together with disulfide bonds. The main types of immunoglobulins and their properties are shown in the table below.

	IgG	**IgA**	**IgM**	**IgD**	**IgE**
Major characteristics	Most abundant internal Ig	Protects external surfaces	Very efficient against bacteria	Mainly lymphocyte receptor	Initiates inflammation Raised in parasitic infection Causes allergic symptoms
Antigen binding	++	++	++	++	++
Compliment fixation	++	-	+++	+	-
Cross placenta	++	-	-	-	-
Fix to mast cells and Basophils	--	-	-	-	++
Bind to macrophages and polymorphs	+++	+	-	-	+

T lymphocytes ands defense against intracellular organisms

Viruses and many small microorganisms enter into the host cells thereby shielding themselves from the attack of antibodies. The body therefore has evolved mechanisms to overcome this threat through the T lymphocytes. These lymphocytes has got special receptors which can recognized and bind to certain peptides derived form the intracellular organisms and integrated to the major histocompatibility complex. When this binding happens the infected cells become target for destruction mechanisms by the T lymphocyte.

T helper cells, T cytotoxic cells and natural killer cells all play an important role in destruction of intracellular organisms by helping macrophages, releasing microbicidal substances, inhibiting replication of viruses and other cytotoxic activities.

Defense against large parasites

Larger parasites cannot be engulfed by macrophages. Instead these parasites are attacked by an antibody dependent cell-mediated cytotoxicity. Antibodies bind to the surface of the parasite and the effector cells bind to these antibodies and initiate an attack on the parasite. Major cell types having a role in this process are the macrophages, the eosinophils and the natural killer cells.

Defense at the mucosal surface

Attachment to the host cells is a pre-requisite for infection. This attachment is made difficult by the innate immunity mucous. But there is a role for the antibodies too. IgA is secreted by the lymphoid tissue and transported to the mucosal surface. This immunoglobulin binds to the microorganisms and makes it more difficult for them to bind to the host cells. This binding also makes the pathogens a target for macrophages. There are also Mast cells coated with IgE at the submucosal surface who are ready to attack pathogens who have passed the mucosa.

Entry, exit and transmission of pathogens

To be able to cause infection, microorganisms must either attach to or penetrate the body surfaces i.e. skin and mucous membranes. There are usually specific molecule on the pathogens that bind to specific receptors on the surface of the host cells. Receptors may be therefore a signs of cell susceptibility for infection. After binding to the cell, the microorganism my stay on the cell surface or enter the cell e.g. viruses and Chlamydia.

The microorganism must also be able to leave the infected host in order to be transmitted to another host. This happens either by shedding in large numbers in body secretions and excretions or by being taken up from the blood by sucking arthropods.

Sites of entry

Skin

Many pathogens can enter the body through the skin whereby they can casue infection in the site of entry in the skin or elsewhere in the body. Microorganisms, apart from the normal flora, cannot resist for long the adverse effects of the low skin PH and susbstances produced by the sebaceous glands. Wounds, abrasions and burns are more common sites of entry than normal skin. Even tiny abrasions can become a site for entry of pathogens such as hepatitis B and streptococcus if they are present. Leptospira and larvae of schistosoma and anchylostoma can traverse the intact skin.

Microorganisms that can enter and infect thru the skin directly include Streptocooci (impetigo, erysipelas), Staphylococci (boils), wart viruses, Rickettsia(typhus), Tricophyton (ringworm), anthrax bacillus, Treponema pallidum (syphilis) and larva of anchylostoma. Others that enter thru bites of animals and arthropdes include rabies virus (rabies), plasmodium(malaria) and arboviruses.

The conjunctiva

Because of the cleansing effect of tears, the rubbing effect of eyelids and the microbicidal effect of lysozeme, very few microorganism can attach an to the conjunctiva and infect it. These pathogens such as Chlamydia and gonococci must have specific attachment mechanisms to the conjunctival cells.

Respiratory tract

It is estimated that one cubic meter of air inside buildings contains 500-1000 microorganisms. In this way every day thousands of pathogens are taken in with respiration. Majority of these will be trapped by the mucus and pushed up to the back of the throat by the ciliary action and swallowed. Microorganisms that enter the lungs will be attacked by the alveolar macrophages. Pathogens that are able to overcome these defensive mechanisms can infect the lungs.

Some microorganisms have specific attaching molecules called adhesins that bind to specific receptors on the susceptible cells of the mucociliary sheet. Other pathogens such

as B. pertussis can interfere with the action of cilia thru production of ciliostatic substances or other unknown mechanisms.

A variety of microorganisms can enter and infect humans thru the respiratory tract such as influenza viruses (influenza), rhinoviruses (common cold), Streptococcus, H. influenzae and Mycoplasma pnemoniae (pneumonias), measles virus and others.

Gastrointestinal tract

Flow of intestinal contents and their excretion is considered a way of cleansing bacteria. Vomiting and diarrhea may also be considered in this context. Under normal conditions the multiplication of bacteria in the intestine is counteracted by their expulsion with feces. If bacteria are to infect the intestine, they must avoid this action by attaching themselves to the mucosa and multiplying. Vibrio cholerae, rotaviruses and salmonella have this ability. The fact that these organisms infect mainly the small intestine or the large intestine indicates presence of specific receptors in the mucosal cells of these tissues.

Parasites have more simple mechanisms to avoid being washed away with the intestinal contents. Giardia has got specific molecules to attach to the microvilli of the epithelial cells. Hookworms attach to the mucosa by a mouth capsule with hooked teeth. Ascaris maintains its place in the lumen by positioning itself against the peristalsis. Trichuris penetrates the mucosa to the deeper layers.

Microorganisms that infect the gust must be able to survive the effect of acid, proteolytic enzymes and bile but majority of bacteria prefers slightly alkaline conditions. The M cells in the Pyer's patches can uptake microbial endotoxins, exotoxins and proteins from the lumen and deliver them to the underlying immune cells for neutralization.

Urogenital tract

The urogenital tract is a continuous passage so microorganisms can easily pass from one part to another. Urine has a flushing mechanism to expel bacteria. Duirng adult life, the vaginal epithelium contains glycogen which is fermented to lactic acid by the Doderlein bacilli. This give vagina a low PH around 5 which prevents colonization of microorganisms other than the normal flora.

Urogenital invasion always occurs from the exterior via urethra and the invaders must be able to attach to the mucosa at avoid being flushed away by urine. Gonococci and Chlamydia attach to special molecules on the surface of the epithelial cells. The pathogen-bound cells induce macrophages to engulf them thereby initiating the infection. Urinary tract infections are rare in males because of the long flaccid urethra (20 cm) but they are more common in females due to the shorter urethra (5 cm) and its vicinity to the anus. It is estimated that UTI is 14 times more common in women.

Oropharynx

The flushing action of saliva provides a cleansing mechanism other defense mechanisms include the microbicidal substances of saliva, the normal flora, secretary IgA and the leukocytes present in the saliva and mucosa. Invasion of oropharynx requires attachment to the mucosa or tooth surfaces. Factors that reduce mucosal resistance allows foreign and commensal bacteria to invade. Vitamin C deficiency makes the gums more fragile and susceptible to infection. Reduction of saliva for a few hours such as between meals leads to

a four fold increase in the number of bacteria in the saliva. In dehydrated persons there is huge overgrowth of oral bacteria.

Exit and transmission

Exit from the body of the infected host is essential for transmission to other hosts. Even the most lethal pathogens will not have a big effect on the host population if they don't have successful exit and transmission strategies. Almost all organisms leave the body through body surfaces. A few are taken from inside the body by blood sucking vectors. Successful transmission of pathogens depends on the folloing factos:

- Number of microorganisms shed from the host
- Number of microorganisms required to infect the new host
- Stability of the microorganism in the environment

The more the number of the organisms shed, the more is their chance to get transmitted to a new host because the majority of them die outside the host. Microorganisms that can resist drying can spread more easily. Certain organisms have developed special resistant forms such as spores of clostridium and cysts of ameba that help them remain for longer periods in unfavorable conditions. The infective dose of pathogens differs for examples 10 shigella organisms can cause infection whereas 1 million salmonella are needed to infect a person. This infectivity also depends on the nature of the microorganism and the route of entry. Direct inoculation to the blood and tissues with a small dose will cause infection.

Certain symptoms of the infection may help transmission of the pathogen to other hosts. For example coughing, sneezing and diarrhea all help cleanse the body of the host from the microorganisms and from this perspective they are useful to the host. But these symptoms are beneficial to the pathogen because they help its transmission.

Routes of transmission

The commonest infections are transmitted through the respiratory and gastrointestinal tracts. Other routes include urogential tract, oropharynx, skin, blood, milk, and bite of animals.

1) Respiratory tract

Respiratory infections usually cause increased mucous secretion, cough and sneezing which is and effective way of shedding microbes. Droplets contain hundreds of microorganisms. The size of the droplets determines their fate; the larger ones travel only few meter and settle on the ground but smaller ones (1-4mm) remain suspended in the air. Overcrowding increases risk of respiratory diseases because of ease of droplet transmission in such conditions. In winter people tend to remain near to each other in closed rooms which is why these infections are commoner in winter. Air conditioning also plays a role by drying the mucosa and making it more susceptible.

2) Gastrointestinal tract

Gastrointestinal infection are related to public health measures and poor hygiene because the microbes need to travel from the feces of infected people to the mouth of new hosts. Use of toilets, proper sewage disposal, hand cleanliness and food hygiene make this transmission more unlikely.

3) Urogenital tract
Microorganisms are shed with urine and mucus and transmission is usually as a result of mucus contact mainly from sexual contact. Therefore sexually transmitted diseases (STD) are the commonest among these infections. Gonococcus and Chlamydia induce mucus discharge and they are transmitted thru mucus contact. Others like syphilis and herpes cause sores and transmission is via the mucosal ulcers. Semen may contain HIV and Hepatitis B viruses and transmit them to the partner.

Transmission of STDs depends on patterns of sexual activity. Promiscuity is essential for spread of these infections and having multiple partners increases the risk of infection. Condoms can retain gonococci, herpes simplex, HIV and Chlamydia.

The female genital tract can also be a source for some newborn diseases through direct contamination during descent thru the birth canal. Conjunctivitis, pneumonia and bacterial meningitis can happen in this way.

4) Oropharynx
Saliva may be the vehicle for transmission. Streptococci and tubercle bacilli may reach saliva from upper and lower reparatory infections. Some viruses such as cytomegalovirus and herpes simplex may infect the salivary glands and spread in this way. Young children may contaminate objects or their fingers with their saliva and spread or get infection in this way.

5) Skin
Skin can be a source of transmission either by shedding the microorganisms to the environment or by direct contact with the infected sites. Dermatophytes(ringworm fungi) are shed from the infected skin, nails hair. Streptococci and staphylococci can spread from skin lesions (boils, carbuncles, impetigo) by direct contamination of the skin and fingers. Viruses such as herpes simplex, zoster, coxackievirus and papilloma viruses can infect the skin and spread thru skin lesions. Some parasites such as Lesihmania tropica and sarcoptes scabiei can also infect the skin.

6) ***Milk***
Microorganisms are rarely shed from human milk except some viruses such as mumps, HIV and cytomegalovirus. Milk of animals may be a source for salmonella, mycobacterium, brucella, staph and streprococci.

7) Blood
Many microorganisms may be picked up from infected blood by sucking arthropods, needles and during blood transfusion. Blood is also source of transplacental transmission.

8) Vertical transmission
This is transmission of microorganisms from parents to offspring via sperm, ovum, placenta, milk or blood. Vertical transmission can be germline, placental, perinatal pr postnatal. Many retroviruses are known to maintain themselves vertically in successive generations of mice. There are also many retroviral DNA sequences in human genome but these are not capable of causing infection. Rubella, cytomegalovirus, syphilis and toxoplasmosis can cross the placenta to the fetus. Gonococcal and chlamydial conjunctivitis may happen perinatally form contamination from the birth canal. Cytomegalovirus and HIV can spread postnatally to the child through breast-feeding.

9) Transmission from animals

Close contact with vertebrate and invertebrate animals(insects and arthropods) puts us in risk on many infections. Invertebrate vectors include mosquitoes, flies, ticks and mites. Insects may carry pathogens on their mouth parts and bodies. Diarrhea and trachoma may spread in this way. Blood sucking insects act as biological vectors and transmit many diseases such as yellow fever, hemorrhagic fever, plague, typhus, leishmaniasis, trypanosomiasis, malaria and filariasis.

Other invertebrates such as shelfish can passively carry vibrio cholerae and viruses and transmit it when the shelfish is consumed as food.

Many pathogens are directly transmitted to us from vertebrate animals. Zoonosis indicates any infection we get from vertebrates whether directly from eating or indirectly via a vector. Anthrax, brucellosis, leptospirosis, salmonellosis, TB, ringworm, giardiasis, toxoplasmosis, toxocariasis and many helminthes may be tranmsited to us from mammals and birds.

Parasite survival strategies

The most common infectious agents have some sort of "awareness" to the host defense mechanisms and have developed certain answers to them. The pathogens can respond to the innate defenses by avoiding being killed by the phagocytes, by killing the phagocytes and by interfering with the ciliary action. But more complicated are the strategies that certain microorganisms have evolved to evade the adaptive defenses on the host.

These strategies are more important for the microorganisms because many of them can avoid the innate defenses but they cannot hide themselves from the immune system. For example many bacteria have the polysaccharide capsule which prevents non-immune contact with the phagocytes but it is readily recognized by the B cell surface receptors. Intracellular organisms also are kept away from phagocytosis but their peptides are presented on the host cell wall and thus recognized by the immune system.

Survival strategies can taken many forms and last for variable periods of time. Certain microbes are able to undergo a lengthy period of growth during the pre-clinical period such as Hepatitis and hepatitis. Others start spreading to new hosts before the onset of the clinical symptoms such as measles. Mycobacteria can persist for years in the host without apparent disease and get reactivated any time during life of the host. Others can continue being shed from the host even after recovery such as typhoid.

Viruses are particularly efficient in evading the immune system for a number of reasons:

- Their invasion of cells and tissues is usually silent. They do not produce toxins and they don't cause extensive cell destruction.
- Some viruses such as rubella, wart viruses, hepatitis B and EB virus can infect cells for a long time without affecting cell viability.
- Viruses form intimate molecular relationship with the infected cells. Their replication depends on cellular machinery and they can interfere with production of interferons.

The major strategies used by microorganisms to evade the host immunity include concealment of antigens, antigenic variation and immunosuppression.

1) Concealment of antigens

Some parasites can hide themselves from being detected by the immune system. Hiding places include interior of the host cells and particular sites in the body where lymphocytes do not normally circulate.

Hiding inside the cells

If a microbe can remain inside a host cells without showing its antigens, it will remain unrecognized by the lymphocytes. Even in these situations there will be specific antibody and T cell responses but the pathogen in side the host cell will not be affected. Persistence of Herpes simplex virus in sensory neurons is in this way. During reactivation the immune response will be heightened and the virus will be exposed to it.

Other viruses such as HIV and coronaviruses, display their proteins secretly on the walls of intracellular vacuoles instead of at the cell surface and bud into these vacuoles. Adenoviruses use another strategy by combining one of their proteins to the MHC molecules and preventing these molecules from passing to the cell surface so that the infected cells are not recognized by the cytotoxic T cells.

Colonizing in places out of reach of lymphocytes
Microorganisms that colonize on the skin and on the intestinal and other mucosa and those shed in the secretions, are out of reach of circulating lymphocytes. These microbes are still under attack of secretary antibodies which can render the microbes less effective but usually are unable of destroying them.

It is more difficult to avoid the lymphocytes and antibodies within the body but there are areas which are safer for the pathogen such as the central nervous system, joints, testes and placenta. These areas are less accessible by the lymphocytes, antibodies and complement. However once an inflammatory response is initiated these areas also lose their safety.

Some parasites create their own protected site such as done in hydatid disease where the tapeworm Echinococcus granulosus forms a cyst around its colonies to protect them from the circulating antibodies. The most highly protected site for parasites is host DNA which can be occupied by retroviruses such as HIV. The retroviral transcribed DNA (from its RNA) is integrated to the host cell DNA making the virus totally anonymous. If this integration happens in the ova or sperm cells the virus becomes part of the genetic material and inherits from one generation to another. Fortunately this does not happen with wild viruses such as HIV. However retroviral DNA sequences are integrated to human genome but they are not expressed as antigens.

Mimicking host proteins
One method of avoiding an immune response is by mimicking host antigens. There are many example of parasite molecules which resemble those of the host. The most famous example is the cross reaction between group A *B*-hemolytic streptococcus and human myocardium. This cross reaction underlines development of rheumatic heart disease from effect of antibodies made against the cross-reacting molecule meromyosin.

Taking a cover made of host molecules
Schisrtosoma acquires a complete surface coat of host glycolipids, MHC antigens and immunoglobulins from the plasma. In such a situation the parasite is completely invisible to the immune system. This strategy is restricted to worms.

2) Avoiding induction of an immune response
If the microbe can avoid inducing an immune response in the host this will be beneficial to it. The microorganism can do this in several ways. Infection during early embryonic life is one way. Intrauterine infection of rubella, cytomegalovirus and syphilis eventually leads to IgM production by the fetus but cell-mediated responses are more significantly impaired. Consequently it takes years to clear the microbes form the body. Neonatal infection with hepatitis B frequently results in permanent carriage of the virus.

Another way of inducing immune tolerance is by producing large quantities of antigens and antigen-antibody complexes such happen sin disseminated coccidomycosis, cryptosporidiosis and visceral leishmaniasis.

2) Antigenic variation

Another way of evading the immune system is by changing the "appearance". Many viruses, bacteria and parasites undergo this change in their antigens either during the course of infection in the same individual or during their spread in the community among different individuals. There are three main mechanisms for antigenic variation; mutation, recombination and gene switching.

Mutation

Influenza virus is famous for its antigenic variation. As it spreads in the community it changes it undergoes repeated mutations in the genes coding for hemaglutinin and neuraminidase. These mutations cause cause small changes that are sufficient to reduce effectiveness of the B and T cell memory built up in the previous infections. This antigenic drift is also shown by rhinoviruses and enteroviruses. Antigenic drift may also be responsible for presence of many antigenic types of streptococcus and staphylococcus.

Recombination

Recombimation happens when exchange of genetic material happens between two different microbes which leads to sudden and considerable variation. The classic example is influenza A virus in which human and avian strains recombine a new strain of influenza A.

Gene switching

Gene switching was first noticed in African trypanosomiasis. T. gambiense and T rhodesiense carry a variety of gene for numerous surface antigens. The parasite can switch from using one gene to another. Gene switching is also thought to be responsible for relapsing of some infections such as brucellosis and relapsing fever.

3) Immunosuppression

Many microorganisms cause immunosuppression in the infected host. Many viral infections cause a temporary weakness in immune response during which period the virus can grow, spread and shed before being eliminated by the host. HIV is unique in its general and lasting immunosuppressant effect.

Diseases that are associated with Immunosuppression usually involve infection of specific immune cells such as infection of the T cells (measles, HIV), Infection of B cells(EBV), macrophages(HIV, leishmania). This cell infection may result in impairment of cell function and division; blocking of release of cytokines and interluekins; and cell death.

There are other methods by which microorganisms can weaken host immune response. HIV releases immunosuppressive molecules such as eg41 polypeptide which acts as an immunologic anesthetic. Poxviruse, herpesvirus and T cruzi release substances that interfere with the action of compliment and cytokines. Staphylococcus exotoxins interfere with the immune system by killing T lymphocytes; inducing excessive liberation of cytokines by immune cells causing upset of immune balance; and polyclonal activation diverting T cells into ineffective immune activities. N gonorrheae, S pneumoniae and H influenzae do not interfere with development of immune substances and cells but liberate a protease capable of cleaving human IgA. Some virulent staph inhibit phagocytosis of antibody-coated bacteria. An elastase released by Pseudomonas can inactivate some complement components.

Persistent infection
Sometimes the host immune fails to control growth and spread of the pathogen and eliminate it form the body resulting in persistence. Persistence can take several forms

- Infectious form: Hepatitis B, HIV and schistosomiasis persist in blood and in blood vessels.
- Non-infectious form: herpes simplex persists in sensory neurons in the dorsal ganglia. Mycobacterium persists in the lymph nodes and lung tissue.

Persistent latent infections are important because they can be reactivated, they may be associated with chronicity and cancers such as hepatitis B virus(hepatocellular carcinoma) and EBV (Burkitt's lymphoma and nasopharyngeal carcinoma). Reactivation occurs in immunocompromised persons such as chronic diseases, AIDS, immunosuppressive therapy, pregnancy and old age.

Persistent infections					
	Microorganism	**Site of persistence**	**Infecti-ousness**	**Consequence**	**Shedding to outside**
Viruses	Herpes simplex	Dorsal root ganglia Salivary glands	- +	Activation, cold sore Not known	+ +
	Varicella zoster	Dorsal root ganglia	-	Activation, zoster	+
	Cytomegalovirus	Lymphoid tissue	-	Activation, disease	+
	Ebstein-Barr virus	Lymphoid tissue Epithelium Salivary glands	- -	Lymphoid tumor Nasopharyngeal cancer Not known	- - +
	Hepatitis B	Liver, blood	+	Chronic hepatitis, liver cancer	+
	Adenoviruses	Lymphoid tissue	-	Not known	+
	T cell leukemia virus	Lymphoid tissue	+-	Leukemia	-
	HIV	Lymphocytes, macrophages	+	Chronic disease	+
Chlamydia	C. trachomatis	Conjunctiva	+	Chronic conjunctivitis	?
Rickettsia	R prowazeski	Lymph node	?	Activation	+
Bacteria	Salmonella typhi M tuberculosis Trep pallidum	Gall bladder, Urine Lung, lymph nodes Disseminated	+ ? +-	Carrier Activation, TB Chronic disease	+ + -
Protozoa	Plasmodium vivax Toxoplasma gondi Trypanosome cruzi	Liver Lymph, muscle, brain Blood	? +- +-	Activation-malaria Activation Chronic disease	+ - -

Pathologic consequences of infection

Invasion of the body by microorganisms may results in various symproms in the host. These symptoms may be caused by the microorganism or by the host immune response. Symptomes that appear rapidely are usually die to direct action of the microorganism and its toxic products. Other pathologic changes are secondary to the immunologic mechanisms both the natural and the adaptive system.

1) Direct effect of the microorganism and its toxins

Direct effect

Organisms that multiply inside cells usually cause rapture of the host cell and spread in this way. Many viruses, some bacteria and some intracellular protozoa behave in this way. Other direct effects of infection include blockage of hollow viscera by worms, blockage of lung alveoli by heavy growth or infective agents such as pnuemocyctis carinii; and direct mechanical effect on host issues such as by hydatid cyst.

Exotoxins

Some parasites especially bacteria produce exotoxins which may cause serious tissue damage. Most exotoxins are proteins. Some bacterial toxins are composed of two subunits; one of which is responsible for binding and entry to the host cell and the other responsible for the toxic effects. Some of the bacterial toxins have been inactivated by formaldehyde without altering their antigenicity and the resulting toxoid is used as vaccines.

Microbial toxins act by various mechanisms to damage the host cells and tissue:

- Some bacteria produce enzymes that break down cells and intracellular tissue allowing spread of infection. Examples: hayaluronidase, collagenase, DNAase, streptokinase.
- Some toxins destroy the integrity of the cell membrane by enzymatic action. These toxins are called hemolysins and produced by Streptococci, Staphylococci and pseudomonas.
- Some toxins enter the cells and alter cellular machinery. Thes etoxins have two subunits: A subunit is the active component and B subunit which is responsible for entry. Once A subunit is inside the cell it is activated and disturbs cellular mechanisms in different ways.
    - Diphtheria toxin blocks protein synthesis
    - Cholera toxin disturbs the sodium/chloride transport across the cells leading to water and electrolyte loss. E. coli and salmonella toxins work in a similar way.
    - Tetanus and botulism toxins interfere with neuronal impulse transmission leading to spastic paralysis in tetanus and flaccid paralysis in botulism.

Endotoxins

Endotoxins are integral parts of the microbial cell wall which are only released after the bacteria dies. Endotoxins are characteristic of Gram negative bacteria and they are usually composed of lipopolysaccharide. The clinical effect of endotoxins are fever, vascular

collapse and shock. Endotoxin shock usually occurs in septicemia with E. coli and N. meningitidis. Disseminated intravascular coagulation is a very sever but rare effect of endotoxins. Some of the effects of endotoxic shock are due to release of cytokines especially interleukin and tumor necrosis factor by the macrophages.

2) Pathologic consequences of the immune response

Infection may lead to overreaction of the immune system or hypersensitivity. Hypersensitivity of microbial origin may be of any of the four types. Many organisms can cause hypersensitivity but it is more common with prolonged infection with continuous and repeated antigenic stimulation.

Helminthes cause type I (allergic) hypersensitivity, viruses, malaria and many bacteria may cause type II (cytotoxic) and type III(immune-complex mediated) hypersensitivity. TB, leprosy cause type IV(cell-mediated) hypersensitivity.

It is thought that skin lesions of many infectious diseases such as measles, syphilis, scarlet fever leprosy, chickenpox have immunological basis. The mechanism of these lesions may be cell-mediated or immune complex-mediated hypersensitivity. The rash is thought to be part of the body's defense against the pathogen.

3) Viruses and cancer

Some RNA and DNA viruses can cause permanent malignant changes within the cells. Example of these tumor viruses include

- Human T cell lymphotropic virus is associated with lymphoma and adult T cell leukemia
- EBV is associated with nasopharyngeal carcinoma (south asia) and Burkitt's lymphoma(Africa)
- Human papilloma viruses are associated with cervical cancer and possibly with squamous cell carcinoma
- Hepatitis B virus is associated with hepatocellular carcinoma

Diagnostic techniques in microbiology

Frequently the initial diagnosis of infection is based on clinical signs and symptoms. Confirmation on many bacterial and fungal infections has been largely based on isolation of the organism from patient specimens. Other diagnostic methods are culture and serological tests.

1) Non-cultural techniques
These techniques try to identify microorganism without cultivation to multiply the microorganism before detection. Some of these techniques such as direct microscopy can be very rapid and practical and provide results in few hours. Others such as polymerase chain reaction may require more than one day.

Light microscopy
Light microscopy has a fundamental role in microbiologic diagnosis because majority of pathogens are not visible to the naked eye. Light microcopy can be used directly on wet specimens or on specimens after staining with different dyes. Protozoa and helminthes are usually identified directly without culture or staining.

Wet preparations are used to demonstrate

- Blood cells and microbes in fluid specimens such as urine, feces and cerebrospinal fluid
- Cysts, eggs and parasites in feces
- Fungi in skin
- Protozoa n blood and tissues

Staining techniques are used to demonstrate cells and microorganism so that hey can be seen more easily. Stains are usually applied to specimens that are dried and fixed on a slide. Microbial cultures can also be stained. The stained specimen is viewed by light microscopy. Oil immersion lens improves the resolving power of the microscope.

The most widely used staining method is **Gram stain** which depends on the differential properties of the microbial cell wall in regard of uptaking stains. Based on reaction to Gram stain, bacteria can be divided into Gram positive which show purple after staining and Gram negative which show pink after staining.

Another staining method for bacterial detection is the **Ziel-Nelseen stain**. Some bacteria like Mycobacteria do not uptake the Gram stain. The Ziel-Neelsen procedure uses heat to drive the fuchsin stain into the cells. Mycobacteria stained with fuchsin stain resist decolorization with acid and alcohol and they are therefore called acid-fast or alcohol-fast. Other bacteria lose the color when subjected to acid or alcohol.

Direct microscopy can be used with adaptation of the amount of light that illuminates the specimen to improve visualization. Thus **dark field illumination** can show the microorganism brightly against a dark background and this method is usually used to demonstrate the motility of microorganisms. It is also used to visualize very thin cells such as spirochetes because the light reflection from the surface of the cell makes them look larger they really are.

Fluorescence microscopy is used to visualize fluorescent substances. Some biological substances are naturally fluorescent, others can be stained with fluorescent dyes and viewed in a microscope with an ultraviolet light source in stead of white light. Fluorescent microscopy is widely used in microbiology and immunology to detect microbial antigens after staining with specific antibodies tagged with fluorescent dyes(immunofluorescence).

Electron Microscopy
The electron microscope uses a beam of electrons instead of light, and magnets are used to focus the beam instead of lenses used in the light microscope. The whole system works in a vacuum. The specimen needs to be cut into very thin sections through which the beam passes and produces the image on a fluorescent screen. The images are photographed and enlarged so that here is many thousand fold magnification of the specimen.

Electron microscopy is used to visualize intracellular structures, microbial antigens and toxins, viruses and virus particles.

2) Culture of microorganisms
Bacteria and fungi can be grown on slid nutrient media to produce colonies composed of thousands of cells derived from a single cell implanted on the agar surface. The colonies of different species of microorganisms grow into characteristic shapes that give a clue to identification of the bacteria. Culture usually requires 1-2 days to grow enough colonies to make them visible but some bacteria grow slowly and may require weeks to become visible.

Cultures can also be made in liquid media (broth) and bacterial growth is then detected by observing turbidity in the medium. But we cannot tell whether one species or more has grown in the broth and whether the organisms are numerous or not. So solid media culturing is more useful for diagnosis.

There are few pathogens that can only be cultured in experimental animals such as M. leprae and T pallidum Some bacteria. There are others that cannot be cultured on artificial media such as Chlamydia and rickettsia; these can be grown in cell cultures. These culture cells are usually derived from human or animal cells adapted to grow in vitro and stored at -80 degrees C until required.

Many culture media are designed to support growth of certain pathogens and inhibit growth of others. These selective media are used to separate pathogens form non-pathogens and normal flora which may be present in the specimen. Parasites such as Leishmania, trypanosoma and trichomonas can be grown in liquid cultures to increase their numbers in the specimen and facilitate identification.

When bacteria are cultured, identification is done on pure cultures or single colonies. Aseptic techniques must be used to keep the cultures clean. Bacteria can be identified on the following basis:

- Gram stain
- Morphology and grouping of cells
- Growth under aerobic and anaerobic conditions
- Growth requirements

- Ability to produce detectable enzymes such as catalase, c oagulase
- Ability to metabolize sugars by oxidation or fermentation

Fungi are identified on the basis of cell morphology and colony characteristics.

Antibiotic sensitivity test can be performed on the pure cultures by exposing the organism to antibiotics contained in filter paper discs.

Viruses can be identified by their cytopathic effect in cell cultures and their morphology in electron microscopy but diagnosis is more often made by serologic tests.

3) Serologic methods

Antibodies usually take several weeks to form and become detectable in he serum of the patient after infection therefore serologic tests that depend on antibody (IgG) detection are retrospective. A positive antibody test indicates that he person has come in contact with the infection in the past. IgM antibodies may be detectable earlier after one week.

Despite this antibody detection methods are very useful for diagnosis of infection with those pathogens that cannot be cultured or can only be grown with difficulty such as viruses. Several infections can be screened simultaneously. Previous immunization makes it difficult or impossible to interpret serologic tests. Example of serologic tests are antigen-antibody precipitation methods, compliment fixation test, enzyme-linked immunosorbent assay(ELISA) and immunofluorescent assay.

4) Genome detection methods

These methods are based on polymerase chain reaction which is a sensitive way for viral genome detection. The viral genome is amplified to a detectable level by using specific primers that bind to the viral RNA or DNA. These tests are especially useful when diagnosis is difficult with other methods, they can find very low numbers of proviral DNA in blood and they can also be used to quantify the viral load in the blood by measuring cell-free viral RNA or DNA. However these tests are expensive, time consuming and require technical skills.

Vaccination

Vaccine is an immunologic substance designed to produce specific protection against a given disease by stimulating the production of protective antibody and other immune mechanisms. Vaccination aims to prime the adaptive immune system to the antigens of a particular microbe so that a first infection induces a secondary response. The principle of vaccination is therefore to induce a state so that when the body comes into contact with the relevant microorganism, it mounts an effective secondary immune response leading to the prevention of the disease.

The British physician Edward Jenner (1749-1823) is considered the founder of vaccination. He had noticed the resistance of milkmaids to infection with severe forms of smallpox. His famous experiment was inoculation of an 8-year old child with liquid from the pustules of a lady with infected with cowpox. Subsequently he inoculated the child with liquid of smallpox lesions and the child failed to develop smallpox. Following this successful experiment he performed this inoculation on thousands of people in his house.

The ultimate aim of vaccination is eradication of the disease though till now only smallpox has been eradicated thanks to vaccination. Eradication of diseases depends on many factors related to the host, parasite and the environment. However while vaccination has led to dramatic reductions in the occurrence of many diseases in the community, the main focus of immunization remains the protection of the individual from the infection.

Properties of vaccines

A good vaccine must be effective against the disease, safe, stable and cheap. An **effective** vaccine must not only induce an adequate immune response but the response must be of the right type. For example a purely antibody response will not be of benefit against tuberculosis and a purely cell-mediated immune response will not be effective against pneumonia. Effective vaccines must also produce an immune response for a good duration of time. Generally living vaccines induce a stronger and more lasting immunity.

Safety of vaccines is also a major consideration because they are given to healthy people and to millions of them so nay possible adverse effects will affect many people and impact negatively on vaccine industry. Fortunately in view of the hundreds of millions of doses that are given annually around the world, vaccine safety is quite good.

As vaccines may remain for sometime before being used, **stability** is a requirement. Stability is especially important in case of live-attenuated vaccines which must be kept in a cold temperature from factory until use. For example it has been noted that polo vaccine will remain stable for one year at 4 degree Celsius but only for few days at 37.

Cost of the vaccine may limit its accessibility especially in the developing countries. Since vaccines are meant for huge numbers of people, if the cost is high, the use will be highly limited. Some international funds such as WHO and UNICEF are mobilizing funds to ensure vaccines to poor countries.

Types of vaccines
To be able to induce a specific immune response, the vaccine must contain some antigenic materials from the infectious agent. Current vaccines either contain a killed microorganism, an attenuated microorganism, toxins or sub-cellular components.

2) Live attenuated vaccines
Mmicroorganisms are attenuated by passing repeatedly in cell cultures in vitro such as monkey kidney cells or human embryo cells. Another method is adaptation to low temperatures. Current attenuated virus vaccines have been selected by selction of mutants induced in this way. BCG is one notable bacterial attenuated vaccine which was prepared from an attenuated strain of M bovis by Calmette and Guerin after more than 10 years(1908-1918) of culture on Glycol-bile-potato medium. Toxoid preparations are highly effective and safe. Recently an attenuated strain of S typhi was produced by exposure to chemical mutagens. Genetically engineering mutations involving deletion of the virulence genes shows great promises in both bacteria and viruses.

These attenuated microorganisms have lost their pathogenicity but retained immunogenecity. These are more potent than killed vaccines because:

- Organisms multiply in the host and provide a larger antigenic dose
- Have all major and minor antigenic components
- Some vaccines engage tissue at port of entry like mucosal engagement by oral polio.

Protection is usually achieved by a single dose. Live vaccines must be properly stored to keep them effective. They must not be administered to people with impaired immunity. Pregnancy is also a major restriction unless specifically indicated. Reversion to virulence is a possibility.

Examples: BCG, typhoid, oral polio, yellow fever, measles, rubella, mumps, influenza virus, epidemic typhus.

1) Inactivate(killed) vaccines
Living vaccines may not be available because of failure to produce attenuated strains of the microorganism. In such situations killed vaccines are a good solution. Mcroorganisms are killed by heat or chemicals. When injected to the body they stimulate active immunity. Safe but less effective than live vaccines and usually require multiple doses. Killed vaccines are usually given parentrally and frequently a booster dose is required. The only contradiction is hypersensitivity to the vaccine.

Examples: typhoid, cholera, pertusis, meningitis, plague, rabies, Salk polio, influenza, hepatitis A.

3) Toxoid vaccines
The toxins produced by certain organisms are detoxicated u sually be formaldehyde and used as vaccines. These toxoids are no more toxic but they still induce antibody production. The antibodies initiated by the vaccine neutralize the bacterial toxin during infection so the vaccine does not have a direct effect on the organism itself. Two of such toxoids, diphtheria and tetanus are among the most successful vaccines. These two are

combined with killed pertussis vaccine and given as triple vaccine. These is evidence that omission of the pertussis vaccine reduces antibody response to the toxoids. Therefore the pertussis vaccine also acts as an adjuvant to the toxoids.

4) Cellular components

Some vaccines are made of certain immunogenic components of the microorganism such as menengiococcal vaccine from polysaccharaide antigen of cell wall, pnuemococcus vaccine from the capsule and hepatitis B from the polypeptide antigen. Removal of live infectious material is an essential part of ensuring safety of these vaccines.

Complications of vaccination

Contamination of attenuated virus vaccines with other viruses growing in the same cell lines is a possibility particularly when monkey cells are used and there are some monkey viruses which can affect humans.

Hypersensitivity is another complication both in living and killed vaccines. Hypersensitivity may be to egg cell antigens or to the viral antigens. Hypersensitivity may be responsible for the fever sometimes following vaccination. It may happen after measles and mumps vaccination.

Convulsions and brain damage (meningitis and encephalitis) have been reported after pertussis, measles and mumps vaccination (1 in a million). Rubella vaccine may cause arthritis.

Living vaccines are usually contraindicated in immunocompromised people. Administration of such vaccines for people with sever T cell deficiency such as athymic patients and HIV may cause fatal disease. Advantages of live vaccines must be balanced against their potential dangers in immunocompromised people.

The cold chain

A system for storage and transport of vaccines at low temperature from the manufacturer to the vaccination site. Cold chain includes cold rooms, freezers, ice line refrigerators, cold boxes, vaccine carriers and ice packs. Cold chain maintenance is a major concern in the process of vaccination and cold chain failure is a major cause of vaccination failure and waste of resources.

Vaccination schedule

At birth:	BCG, OPV-0, HB-1
6 weeks:	DPT-1, OPV-1, HB-2
10 weeks:	DPT-2, OPV-2
14 weeks:	DPT-3, OPV-3, HB-3
9 months:	Measles
16-24 months:	DPT, OPV
5-6 years:	DPT

Vaccination coverage in Kurdistan

EPI vaccination coverage (by history and card) in children under 5 years of age in Iraqi Kurdistan, 2005*

Vaccine	Sulaimaniyah	Erbil	Dohuk
BCG	99%	98%	97%
Polio-3	77%	64%	64%
DPT-3	77%	62%	67%
Hepatitis B-3	82%	76%	58%
Measles	77%	61%	67%
Fully immunized by one year	69%	51%	53%

* EPI coverage survey October 2005, done by UNICEF and DoH

Passive immunization

In situations where the patient is already infected or thee is immunodeficiency, an alternative to vaccination may be passing immunity to the patient through giving immnoglobulins or antibodies against the disease. The introduction of antitetanus serum in the beginning of the First World War reduced incidence of tetanus by up to 30 fold. The use of antibiotics nowadays has limited the use of passive immunity for only a few diseases such as follows:

- Diphtheria antitoxin (from human, horse): prophylaxis and treatment
- Tetanus antitoxin (from human, horse): prophylaxis and treatment
- Varicella zoster immuneglobulin (from human): treatment in immunodeficiency
- Gas gangrene (from horse): post exposure
- Botulism antitoxin (from horse): post exposure
- Snake and scorpion antitoxin (from horse): post exposure
- Rabies immune globulin (from human): post exposure
- Hepatitis B immune globulin (from human): post exposure
- Hepatitis A immune globulin (from pooled human): prophylaxis
- Measles immuneglobulin (from pooled human): post exposure

The serum which is used for passive immunization may be specific or non-specific and its origin may be human or animal. Specific serum is derived from convalescent patients or after immunization against the specific disease. Use of serum derived from horse or rabbit is largely discontinued because of the risk of sensitivity and even anaphylaxis to the foreign protein. This sensitivity may cause anaphylactic shock or serum sickness due to immune complex deposition in different tissues. Despite this horse antiserum is still given for gas gangrene, botulism and snake bite.

For common infections, non-specific immunity can be provided by administering antibodies in pooled normal serum because it is assumed that most normal people have antibodies against he pathogen in their serum. These immunoglobulins are derived from donated blood of normal people after screening for HIV and hepatitis. This sort of immunization is especially useful for patients with immune deficiency such as hypogamaglobulinemia.

Antimicrobial therapy

Selective toxicity is a term used to denote the toxic effect of the antimicrobial agent on the microorganism without being toxic to the host. This is achieved by exploiting the differences in the structure and metabolism of the microorganisms and the host cells. These differences are more notable between the prokaryotes (e.g bacteria) and the eukaryotes. Ideally the antimicrobial agent should act on a target which is present in the pathogen but absent in the host cells. This selective toxicity is therefore easier to achive for bacteria. On the other hand viruses are difficult to target because they are obligate intracellular organisms. A successful antiviral must be able to enter the host cell and inhibit the virus without adversely affecting the host cell function.

Desirable features of antimicrobials include the following:

- Selective toxicity to the microbe rather than the human targets
- Broad spectrum of activity
- Cidal rather than static effect.
- Non toxic to the host
- Long plasma half life
- Good distribution in the tissues including CSF
- Low protein binding
- Oral and parentral administration
- No interference with other drugs

Antibacterial agents can be natural compounds(antibiotics), derivatives ffrom natural compounds or totally synthetic. Antibiotics are natural metabolic products of bacteria, actinomycetes and fungi that kill or inhibit the growth of other microorganisms. Antibiotic production is associated with the soil microorganism and it is thought to give the producers an advantage in their competition for space and nutrients. Majority of antimicrobials which are in clinical use are derivatives of antibiotics which are modified chemically to improve their antimicrobial activity and their pharmacologic properties. There are others which are totally synthetic.

There are three ways for classification of antimicrobial agents: by chemical structure, by target site and according to their antibacterial activity(whether bacteriostatic or bactericidal).

Some antimicrobials kill bacteria while others inhibit their growth and thus they orevent increase in the number of the bacteria dn the host defense can deal with the static number of the bacteria. Bacteriostatic agents are therefore lss effective in immunodeficient hosts. The distinction is not clear because some agents kill some species and inhibit growth of others e.g. chloramphenicol inhibits E coli but kills H influenzae.

Classification on the basis of site of action is based on the understanding of the mechanism of action of the drugs. There are four main target sites for antimicrobial action.

- Cell wall synthesis
- Protein synthesis
- Nucleic acid synthesis
- Cell membrane function

Classification based on the chemical structure is not of practical use because there are plenty of diversity. Classification based on the target site and chemical structure is more informative.

Resistance to antibacterial agents

Microbial resistance to the antimicrobial agents is variable. In clinical practice, a microbe is considered resistant to the agent if it could not be killed or inhibited by the antimicrobial agent at concentrations of the dug achievable in the body after normal dosage. Some species of bacteria are inherently resistant to some families of drugs either because they lack a susceptible target site or because they are impermeable to the drug. The Gram negative bacilli are less permeable to large molecule than the Gram positive cells. Even within species that are innately susceptible, certain strains may develop acquired resistance.

Acquired resistance has being increasingly recognized as a threat to antimicrobial therapy especially treatment of life threatening infections. Microbial resistance can develop in several ways.

- Chromosomal mutation: a single chromosomal mutation may result in synthesis of an altered protein as in streptomycin resistance. A series of mutations may happen in the chromosome such as happens in penicillin resistant pneumococci. In presence of antibiotics, the mutants will be safe and have the opportunity to outgrow the susceptible forms.
- Transmissible plasmids: some bacteria are capable of obtaining resistance gens from transmissible plasmids. These plasmids code for resistance to several antibiotics at the same time. For example TEM-1 is the commonest plasmid-mediated beta lactamase which is wide spread in E coli and other enterobacteira. It accounts for penicillin resistance in gonococcus and ampicillin resistance in H influenzae.
- Transposomes or jumping genes. Transposomes are capable of shifting between chromosomes and plasmids. Transposomes moving from chromosomes to plasmids allow chromosomal genes to be disseminated more rapidly to other bacteria. Transposomes can alos move between plasmids e.g. from a non-transmissible plasmid to a transmissible plasmid.

The mechanism of resistance can be broadly classified into 3 categories. Firstly, the target site may be altered so that the drug will have lowered affinity to the target on the microorganism. Secondly the uptake of the drug may change so that the drugs will not reach the target site sufficiently. This may happen by reducing cell permeability to the drug or by active efflux mechanism to pump the drug out of the cell. The third mechanism or resistance is inactivation of the drug by enzymatic modification and destruction. Such enzymes include beta lactamases and aminoglycoside modifying enzymes.

Classes of antimicrobial agents

The following sections describe major clinically used antimicrobials according to the target site of action and then according to the chemical structure.

A) Inhibitors of cell wall synthesis
Peptidoglycan is an essential component of the cell wall therefore it is a very good target for selective toxicity. Several groups of antimicrobials inhibit synthesis of cell wall but the important groups are beta lactams and glycopeptides. Cycloserine and Bacitracin have much less clinical uses.

1. Betalactams
Betalactams comprise a very large family of different groups of antimicrobials all of which contain a betalactam ring and inhibit cell wall synthesis by binding to penicillin binding proteins. This group includes

- Penicillins: penicillin, cloxacillin, flucloxacillin, ampicillin, amoxicillin, azlocillin, piperacillin
- Cephalosporins:cephalexin, cefaclor, cefadroxil, cefuroxine, cefixime, cefpodoxime, cefotaxime, ceftriaxone, ceftazidime, cefpirone
- Cephamycines: cefoxitin
- Carbapenems: imipenem meropenem

Many Betalactams are administered orally but majority are parentral preparations. They are well distributed in the body and majority achieve clinically effective concentrations in the CSF in meningitis when the blood brain barrier is more permeable. Betalactams are generally not effective against intracellular organisms.

There are tens of Betalactam drugs currently in **clinical use**. They are used against a diverse variety of microorganisms but they are not effective against organisms lacking a cell wall such as Mycoplasma or having a thick cell wall such as Mycobacteria and intracellular organisms. Penicillins are effective against Gram positive organisms while others are wide spectrum and more recent products are active against Gram negatives and enterobactria.

Resistance to Betalactams may involve one or more of the three mechanisms. Methicillin resistant staphylococci can alter their target site by producing an additional penicillin binding protein which has a lower affinity to the Betalactams than the normal PBP. Some Gram negative bacteria can alter access of the drug to the target site by reduction of the cell membrane permeability to the drug. Betalactamases are enzymes that catalyze the hydrolysis of the Betalactam ring and in this way inactivate the antibiotic. Betalactamazes are produced by both Gram positive and Gram negative bacteria and found on chromosomes and plasmids. Clavulanic acid is a betalactamase inibitor, its molecules contain a betalactam eing which acts as a "suicide inhibitor" binding to the betalactamase and thus preventing destruction of the Betalactam drug.

Toxic effects of Betalactams include mild rashes and immediate hypersensitivity reactions. Serious allergy to betalactam drugs occurs in approximately 4-15 per 100,000 treatment course. Mild reactions are much more common especially with ampicillin. Patients allergic to penicillins are usually allergic to cephalosporins. Benzylpenicillin may cause nuerooxicity if given in high doses and in renal impairment. Carbenicillin can casue platelet dysfunction.

2. glycopeptides
glycopeptides have got large molecules. They interfere with cell wall synthesis at an earlier stage than the Betalactams. For this reasons it is useless to combine them with the

betalactams for treatment of susceptible agents. Examples of this group include vancomycin and teicoplanin. They are not absorbed from the gut and do not cross into the CSF in normal people but does so during meningitis. They are exerted from the kidneys. Both drugs are active against Gram positive organisms only and they are used to treat infections caused by organisms resistant to Betalactams such as staph aureus and staph epidermidis. They are also used to in people allergic to betalactams. Oral admisntration is used to treat Cl difficile enterocolitis. Acquired resistance to these drugs is extremely rare.

Glycopeptides are ototoxic and nephrotoxic. Vancomycine must be given by slow IV infusiuon to avoid "red man" syndrome" from histamine release if given rapidly. Teicoplanin is less toxic and can be given IV or IM.

B) Inhibitors of protein synthesis

Several groups of antimicrobials inhibit protein synthesis by bacteria though the exact mechanisms of this action is not understood but mainly interfering with the function of messenger RNA. These include aminoglycosides, tetracyclines, chloramphenicol, erythromycine and fusidic acid.

1. the aminoglycosides

Aminoglycosides are a family of related molecules that kill and inhibit microorganism. They include gentamicin, kanamycin, amikacin, netilmicin, tobramycin, neomycin , spectinomycin and streptomycine,. They are not absorbed from the gut, do note penetrate well inot tissues and bone and to the BBB. They are excreted from the kidney. Admisntration must be IM or IV. Intrathecal route is used for meningitis. Aminoglycosides are used for the treatment of serious Gram negative infections including those caused by P aeroginosa. They are not effective againt streptococci and anaerobes but active against staphylococcus.

The basic rule in clinical use of aminoglycosides is use only in severe life-threatening infections. Indications include Gram negative septicemia, septicemia of unknown origin, bacterial endocarditis, pyelonephritis, staph aureus septicemia and post-surgical abdominal sepsis. Streptomycine is now reserved for treatment of TB, Neomycine is used for local decontamination and spectinomycin for treatment of betalactam resistant gonorrhea.

Aminoglycosides are ototoxic and neurotoxic and the therapeutic window for successful treatment and toxicity is narrow. Blood concentrations should be monitored especially in renal impairment.

2. Tetracyclines

Tetracyclines are a family of large cyclic structures that inhibit protein synthesis by bacteria but their action is not quite selective to prokaryotes. This group includes many drugs which vary in their pharmacological properties rather than their antimicrobial action. They include tetracycline, chlortetracycline, oxytetracyclne, doxycycline and minocycline. Tetracyclines are usually administered orally and absorbed from the gut but doxycycline and minocycline are better absorbed than he others so they have less inhibitory action on the gut flora. Tetracyclines are well distributed in the tissues, they enter into the cells and inhibit intracellular organisms. They are excreted by the kidneys.

Tetracyclines are active against a wide range of bacteria, but their uses is restricted to wide-spread resistance. They are used for treatment of infections caused by mycoplasmas, chlamydiae, and rickettsiae. Resistance genes are carried on transposomes.

Tetracycones must be avoided during pregnancy and in children under eight years of age. They suppress gut flora resulting in gastrointestinal upset, diarrhea and overgrowth of resistant bacteria. Tetracyclines can interfere with bone development and cause brown staining of teeth in fetus and children. It can also cause liver damage.

3. Chloramphenicol

Chloramphenicol is a relatively simple molecule containing a nitrobenzene nucleus and prevents peptide bond synthesis. It is well absorbed from the gut and well distributed in he tissues, capable of penetrating into into the host cells and to the CSF. It is metabolized in the liver and excreted by the kidney.

Chloramphenicol is active against a wide range of Gram negative and Gram positive bacteria, aerobes and anaerobes including intracellular organisms. But due to rare serious toxic effects its uses is restricted. The main indication of chloraphenicol is against Salmonella typhi. It is also used for treatment of bacterial meningitis particularly H influenzae.

The nitrobenzene component of chloramphenicol is a bone marrow depressant. This toxicity takes two forms

- Dose dependent bone marrow suppression which occurs if the drug is used for long periods and is reversible when the drug is stopped.
- Idiosyncratic reaction causing aplastic anemia which is not dose dependent and is irreversible. It can occur after the treatment has stopped> fortunately it is very rare occurring in about 1 in 30,000 patients treated.

4. macrolides

Erythromycin is the most widely used macrolide. It prevents protein synthesis. Some newer members such as azithromycin and clarithromycin have improved activity pharmacologic properties. Spiramycin is another macrolide used exclusively in the treatment of criptosporidosis and prevention of congenital toxoplasmosis.

Erythromycin is administered orally but can be given IV too. It is well distributed in the body and penetrates into the cells. It is concentrated in the liver and excreted in bile. Erythromycin is used as an alternative to penicillin for streptococcal infection and other Gram positive cocci in penicillin allergic patients. It is also active agnait legionella, camphylobacter, mycoplasma, Chlamydiae and rickettsiae. .

Erythromycin is relative free of side effects. It may cause nausea, vomiting and rarely jaundice.

5. Lincosamides

Lincomycin and clindamycin also inhibit peptide bond formation. Clindamycine is chlorinated derivative of lincomycin and has almost completely replaced it. Clindamycin is usually given orally but parentral use is alo available. It penetrates well to bone but not to CSF. It is metabolized in the liver and several of its metabolites persist in the feces for up to five days after a dose. Clindamycin has a spectrum of activity similar to erythromycin

but it is more active against the anaerobes both Gram negative and Gram positive. However Cl difficile is resistant and it may overgrow in the gut and cause pseudomembranous colitis. Clindamycin is a valuable drug for opsteomyelitis because of its activity against staph aureus and good bone distribution.

6. fusidic acid

Fusidic acid is another drug that inhibits protein synthesis. It is effective against Gram positive cocci and its most important use is for treatment of staphylococcal infections resistant to betalactams and in allergic patients. Resistance is common so it is usually give in combination with another antistaph.

C) Inhibitors of nucleic acid synthesis

Antimicrobials that inhibit nucleic acid synthesis do so by inhibiting synthesis of precursors, inhibition of DNA replication or RNA polymerase. They include sulfonamides, trimethoprim, quinolones and rifampicin.

1. Sulfonamides

Sulfonamides are totally synthesized compounds. The frist sulfonamide was introduced to clinical use in 1935 as the first effective antimicrobial agent. They are given orally often in combination with trimethoprim. Metabolism occurs in the liver and excreted in the kidney. Sulfonamides are active against Grame negatives except pseudomonas and they are useful for urinary tract infections. Resistance is common. They are relatively safe but rashes and bone marrow depression may occur.

2. Trimethoprim

Trimethoprim is one of a group of pyrimidine-like structures similar to antimalarial pyremethamine. It has an action similar to sulfonamides. It is usually iven in combination with sulfamethoxazole (as cotrimoxazole). The advantage of this combination is synergy and avoidance of resistance.

Cotrimoxazole is used for urinary tract infections. It is also active against S typhi and pneumocystis carinii. Side effects include nausea, vomiting and neutropenia.

3. Quinolones

Quinolones are synthetic compounds that prevent supercoiling of the bacterial chromosome. Nalidixic acid is one of the earliest members but there are many new and more effective derivatives. Qunolones are administered orally, well absorbed from the gut and are mostly excreted from the kidneys. Nalidixic acid does not achieve adequate serum concentrations. The newer fluoroquinolones achive significant serum levels after oral doseand are well distributed in the tissues. Nalidixic acid is active only against enterobacteria. Other quninolones such as ciprofloxacin, pefloxacin and norfloxacin are more effective against Gram negative rods. Ciprofloxacin is effective against pseudomonas too. They are used for urinary infections, systemic Gram negative and intracellular infections including S typhi, Chlamydia and rickettsia. They are also used in combination with others for atypical pneumonia, staphylococci, streptococci and enterococci.

Fluroquinolones are not licensed for children due to possible toxic effects on cartridge development. Side effects include gastrointestinal disturbances and more rarely nuerotoxicity and photosensitivity.

4. Rifamycins

Rifampicin is the most common rifamycin. It blocks synthesis of messenger RNA. Other include rifabutin and rifapentine. Rifampicin is administered orally, well absorbed and very well distributed in the body including the CSF. It is metabolized in the liver and excreted in bile. The urine, sweat and saliva of treated patients turn orange which is harmless but good as an indicator of compliance. The primary use of rifampicin is in TB. It is also drug of choice for prophylaxis of meningiococcal and Hemophilus meningitis. Resistance is common and provided by chromosomal mutations. Side effects include rashes, hypersensitivity reactions and jaundice.

5. Metronidazole

Metronidazole is an imidazole with antiparasitic and antibacterial activity through interference with DNA synthesis. It can be given orally or parentrally. It is well absorbed and distributed in tissues and CSF. Metronidazole is effective against trichomonas vagainalis, Giardia, Entameba and anaerobic bacteria. Resistance is rare. Serious but rare side effects include peripheral neuropathy.

D) Inhibitors of cytoplasmic membrane function

Structure of cytoplasmic membrane in bacteria is different from those of mammalian cells and this allows application of some selective toxicity. Some drugs inhibit cell membrane function. Polymixin E (colistin) is the most important member of polymixins. After oral administration it is not absorbed from the gut. It is effective against Gram negative organisms except pseudomonas. It is used for gut decontamination. Topical use for wound dressing and bladder wash. It is not used systemically because of nephrtoxicity.

Combination of antibacterial agents

Combining different antimicrobial agents may lead to synergism or antagonism. Synergism happens when the combined effect is greater than the sum of the two separate effects. Antagonism happens when the effect of one drug is compromised by the action of the other. A widely used example of synergistic combinations is contrimoxazole. Another example is use of penicillin with gentamicin for the treatment of endocarditis caused by enterococci. In clinical practice combinations are used in the following settings:

- To obtain a synergistic action such as cotrimoxazole
- To prevent or delay emergence of persistent infection such as use of isoniazid, refampicin and ethambutol in TB.
- To treat polymicroial infections such as peritonitis
- To treat serious infections in the early stage before the causative agent is identified

Antifungal agents
Selective toxicity is much more difficult to achieve in the eukaryotic fungal cells than in the prokaryotic bacteria. For this reason the number of antifungal drugs suitable for treatment of infections is very limited. Antifungal drugs usually affects the human cells as well as the fungal cells though to a lesser degree. Other problems of antifungal drugs include issues of solubility, stability and absorption.

Antifungal drugs can be classified according to chemical structure and target site of action. Majority of antifungals act on the synthesis and function of the cell membrane except flucytocine and grisofulvin. There are no antifungal drugs inhibiting protein synthesis that are not also inhibitory to human cell protein synthesis.

Azole compounds inhibit cell membrane synthesis through inhibiting an enzyme specific to the fungi. Clotrimazole and miconazle are useful as topical compounds for candidiasis and dermatphytes. Ketoconazole is the drug of choice for many systemic fungal infections such as dermatophytes, coccindiomycosis and histoplasmosis. Fluconazole is usually used for candidiasis. Systemic use of azole compounds may cause anorexia, nausea and vomiting and dose dependent depression of testosterone leading to gynecmstia.

Amphotericin B and nystatin inhibit cell membrane synthesis causing leakage of cellular contents and subsequent cell death. The basis of their selective toxicity is their preference for ergosterol over cholesterol. Nystatin is used for topical treatment. Amphotericin remains the drug of choice for serious systemic fungal infections despite its serious adverse effects such as acute reaction, nephrotoxicity and potassium loss.

Flucytocine and grisofulvin inhibit fungal DNA synthesis. Flucytocine is active only against yeasts and used in treatment of candidiasis and cryptococcosis. Its adverse effects include neutropenia and jaundice. Grisofulvin is a safe drug and used orally in treatment of ringworm(dermatophytes).

There are some other topical antifungal preparations available over the counter such as Whitefield's ointment(benzoic acid and salicylic acid), tolnaftate, ciclopriox, haloprogin, naftifine.

Antiparasitic agents
Many drugs have been developed against parasites. The problem of finding a safe drugs that is not toxic to human cells is a real one because of similarity of parasitic and human cells and complexity and diversity of the parasites and their life cycles. Antiprotozoal and antihelminthic drugs are summarized in the table below.

Therapeutic application of major antiprotozoal drugs		
Disease	Drug	Route
Amebiasis	Diloxanide Metronidazole Tinidazole Dihydroemetine Chloroquine	Oral Oral Oral IM Oral
Cryptosporidiosis	Spiramycine	Oral
Giardiasis	Metronidazole Tinidazole Furazolidin	Oral Oral Oral

Leishmaniasis	Antimonials Pentamidine Amphotercin B	IV/IM IM IV
Malaria	Chloroquine Primaquine Quinine Progoanil Pyrimithamine Mefloquine	Oral Oral Oral, IM Oral Oral Oral
Toxoplasmosis	Pentamidine	IM
Trichomoniasis	Pyrimithamine Sulfadiazine Metronidazole Tinidiazole	Oral Oral Oral Oral
Trypanosomiasis	Suramin Pentamidine Melarsoprol Tryparsamide Nufurtimox Benznidazole	IV IM IV IV Oral Oral
Therapeutic application of major antihelminthic drugs		
Disease	Drug	Safety
Cestodes(tapeworms)		
Adult worms	Niclosamide	Safe
Cysticercosis, hydatid	Benzimidazole	Safe
Trematodes		
Schistosomiasis and intestinal flukes	Praziquantel, oxamniquine	Safe
Liver and lung flukes	Praziquantel	Safe
Nematodes		
Ascariasis and pinworm	Mebendazole, albendazole Flubendazole Pyrantel pamoate	All safe
Hookworm infection	Mebendazole Albendazole Flubendazole	All safe
Strongyloidiasis	Tiabendazole	Milk side effects
Tricuriasis	Mebendazole Albendazole Flubendazole Thiabendzole	All safe
Toxocariasis	Thiabendazole Mebendazole	
Filariasis	Diethyl carbamizine Ivermectin	Mild side effects

Antiviral therapy

There is no specific treatment for the majority of viral infections but there are few effective antiviral drugs such as acyclovir, gancyclovir, vidarabine, idoxuridien, ribavarin, amantadine, and zidovuidine. Most of these interfee with viral RNA/DNA sysnthesis.

Acyclovir is a nucleoside analogue used for herpes infections. Topical preparations is used in genital herpes, herpes dendritic ulcers, cold sores and zoster. Systemic acyclovir is very effective in treatment of herpes encephalitis. It is a safe drug. Idoxuridine is also a

nucleoside analogue which was used to be given for dendritic ulcers but now replaced by acyclovir.

Zidovudine is another nucleoside analogue acting as an inhibitor of reverse transcriptase. It is one of the most useful drugs for AIDS but it is expensive and has many side effects notably bone marrow depression. Zalcitabine(deoxycitidine), didanosine(deoxyinosine) and lamivudine are other reverse transcriptase inhibitors used in AIDS.

Gancyclovir is active against cytomegalovirus and it is used for disseminated CMV infections. Ribavirin is a guanosine analogue which is used as an aerosol for severe respiratory syncytial virus infections and for severe influenza B.

Amantadine has been known since 1960s. it inhibits penetration of the virus into the cells and uncoating of the virus. It is effective against influenza A but not B or other respiratory viruses. It is used for prevention during outbreaks. It is also useful for treatment if given within the first 48 hours of symptoms.

Interferons are very effective against virus replication in vitro. However their clinical use has been disappointing mainly because they have a very short half life in the circulation making I very difficult to achieve adequate concentrations.

Antiretroviral drugs

The pandemic of HIV/AIDS has led to extensive search for safer and more effective drugs for the management of HIV infection. Current antiretroviral therapy regimens are made up of drug combinations. There are three classes of antiretroviral drugs none of which are ideal and all have potentially serious side effects.

1. nucleoside analogue reverse transcriptase inhibitors (NRTI): the first group of drugs developed including zidovidine, abacavir, didanosine, lamivudine, zalcitabine, stavudine
2. non-nucloside reverse trancriptase inhibitors (NNRTI)including nevirapine and efavirenz
3. protease inhibitors (PI) including saquinavir, retonavir, indinavir, nelfinavir and amprenavir.

Common antiretroviral regimens usually include different combinations of these three classes such as a drug from each class or a PI and 2 NRTI or a 2 PI and 2 NRTI. Choice of drugs depends on toxicity, resistance, compliance and previous drug failure.