FACULTY OF HEALTH SCIENCES

UNIVERSITY OF OTTAWA

MICROBIOLOGY

AND

IMMUNOLOGY

HSS 1100

Winter 2012

HSS 1100 LECTURE OUTLINE

SUBJECT

General Principles of microbiology

Pathogenesis of Infectious Diseases

Immunity to Infection

Immunization (Vaccination)

Antibiotic Resistance

General principles of diagnostic microbiology

Gram-positive cocci

Gram-negative cocci

First Midterm Exam (20%)

Gram-positive bacilli

Gram-negative bacilli

Mycobacteria

Spirochetes

Chlamydia, Mycoplasmas

Parasitic Infections

Mycotic (Fungal) Infections

Second Midterm Exam (30%)

General characteristics of viruses

Respiratory Viruses

Enteric Viruses

Viruses causing diarrhea

Viruses causing exanthems

Viruses causing glandular enlargement

Viruses infecting the CNS

AIDS and HIV

Nosocomial Infection and Hospital Infection Control

Cleaning, Disinfection and Sterilization

FINAL EXAM (50%)

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CONTENTS

GENERAL PRINCIPLES OF MICROBIOLOGY

There are micro-organisms almost everywhere in the environment and in association with higher animals and plants. The different classes of organisms generally regarded as micro-organisms and vary widely in their sizes, levels of complexity as well as abilities to grow in different growth conditions.

Basic Structure

Viruses consist almost entirely of nucleic acids and, in some cases, a protein shell known as a capsid. Viruses enter cells and divert the synthetic processes of those cells towards their own replication. In some cases this lulls the cells. Chlamydia are more complex than viruses but still only multiply in living cells. Within the cells they have a recognizable morphology and life history.

Bacteria are **Prokaryotes**, have a rigid cell wall with cytoplasm and their genetic material organized into a circular chromosome. Mycoplasmas are similar to bacteria but do not have the rigid cell wall, and are consequently more delicate.

Eukaryotes - This comprises all unicellular and multicellular animals and plants. The genetic material is organised into a nucleus.

Normal Bacterial Flora

Resident Flora vs. Transient Flora.

The **gut**: In the **colon** there are large numbers of anaerobes and coliform organism. The **skin** is populated mostly by coagulase negative staphylococci.

The ways in which normal flora are clinically important are:

1) A healthy active normal flora will to some extent protect a person from infection by invasive organisms.

2) The normal flora of the gut participates in the metabolism of the host. In particular vitamin K is synthesized by certain microorganisms and if there are disturbances in the normal flora there may be secondary nutritional deficiencies.

3) Disturbances in the ecology and balance of the normal flora may be produced by antibiotic therapy - this is the basis of antibiotic induced diarrhoea.

4) The normal flora is controlled by various host defences (see below) and deficiencies in these defences may result, in some patients, in infections caused by organism which are normally not pathogenic.

5) The presence of normal flora in cultures may confuse the interpretation of laboratory results.

Bacterial Infections and Host Defences

In human health the normal flora bacteria function as a balanced ecosystem with multiple species at a site and no single organism predominating. Organisms do not invade the body outside their normal territory. Infection with bacteria occurs when a single species becomes predominate at a site at which there are normally many species or when a single organism invades a body site which is normally sterile. When this occurs the outcome is determined by the bacterial pathogenicity factors and by the host response to those aggressive bacterial mechanism(s). The most important of these are as follows:

Mechanical Barriers

The skin is a barrier to the invasion of deep tissues by bacteria. The skin is extensively colonized by large numbers of organisms which do not normally gain access to the deeper subcutaneous tissues.

The organisms on the surface of the skin may, however, initiate an infectious process if a break in the integrity of the skin barrier allows them to gain access to the deeper layers.

Adherence

The first requirement for bacterial invasion to occur is that the bacteria will adhere to the host tissues, and bacteria have developed specific mechanisms to achieve this. Ligands are present on the surface of the bacteria which bind to specific receptors on the mammalian cell surface. Adhesion of the bacteria to the host tissue is a prerequisite for the initiation of an infectious process, and takes place before the factors listed above come into operation.

Phagocytosis

Some of the cells of the body, of which the most important are the macrophages and the blood neutrophils (polymorphonuclear cells), are capable of ingesting bacteria and killing them, and are a very important defence against invasion by bacteria. Phagocytosis depends for its initiation on the ability of phagocytic cells to attach to and form a vacuole around bacteria. Some bacterial organisms (e.g., *Streptococcus pneumoniae*) surround themselves with a polysaccharide capsule and can prevent the phagocytic cell from engulfing them. This protects them against phagocytosis. The host response to this is sometimes to produce antibodies to the capsule. The antibody modifies the surface of the capsule in a way that permits the phagocyte to take up the capsulated organism. This is referred to as opsonization.

Antibodies to Bacteria

People may form antibodies to bacteria. Antibodies may function as an opsonin (see phagocytosis above), or may kill organisms directly.

Complement

Complement is a system of plasma proteins that work together to resist bacterial infection. Some bacteria are killed by complement. Complement has two major roles.

Cell Mediated Immunity

Some bacteria such as *Mycobacterium tuberculosis*, *Legionella* species are killed by activated macrophages and cell mediated immunity is a vital defence against these organisms.

Exotoxin Production

Bacteria may produce exotoxins which damage those tissues. The exotoxins are proteins which are synthesized by bacteria and then released, and they may exert their effects at an anatomical location remote from the bacteria which originally synthesized them. The host defence against exotoxins is antibody.

Interactions Between Pathogenicity Mechanisms and Host Defences

The normal flora is held in check, and invasive disease is prevented in the healthy individual by the defences described above. If an invasive infection does occur, the outcome is determined by the interaction between the host defences and the pathogenicity mechanisms of the bacteria.

Metastatic Spread

Bacteria may become distributed around the body from a single focus of infection through the blood stream (bacteraemia or septicemia). When this occurs the sequence of events is firstly that there is a primary focus of infection followed by dissemination of the organisms causing that infection through the blood stream. These organism which have been disseminated by the blood stream then form their own individual first sign of infection at a sight distant from the original infection.

Compromised Patients

Some patients may be deficient in some of their antimicrobial defences, as discussed above. These deficiencies may be due to a disease process (for instance leukemia may give rise to a deficiency in phagocytosis) or to medical or surgical procedures, including the administration of medication. For instance, administration of immunosuppressive drugs to transplant patients may depress their immune system to the point where it is no longer effective as a defence. Below are shown some of the circumstances in which deficiency in host defences may lead to infections. Some host defence mechanisms are more important than others in the prevention of certain specific infections, and hence it is possible to predict which infection will occur in which patient. This is important in the management of infections.

Host Defence Missing	**Patient at Risk for**
Phagocytic cells (leukopenia)	*Staphylococcus aureus* *Streptococcus pneumoniae*
Antibody mediated immunity	*Streptococcus pneumoniae* Viral infections
Cell mediated immunity (includes AIDS)	Tuberculosis *Legionella* All AIDS-related infections

PATHOGENESIS OF INFECTIOUS DISEASE AND THE IMMUNE RESPONSE

BACKGROUND OF MICROBIAL DISEASE

Microbial diseases (infectious diseases) are the result of the interaction between microorganisms (penetrating and multiplying) and the host organism.

1. MICROORGANISMS

Definition: organisms invisible to the naked eye (viruses, bacteria, fungi and parasites).
The majority are harmless, many of them useful; only a very small proportion produce harmful effects in animals and plants.

2. INFECTION

Definition: Penetration of a microorganism, or a part of it capable of multiplication, into a host organism, producing apparent (= disease) or inapparent (= no disease) changes. Infection should not be confused with:

> **Colonisation:** establishment and multiplication in/on the body of a microorganism without producing any apparent or inapparent change (e.g., colonisation of the skin with micrococci).
>
> **Contamination:** deposition of microorganisms without multiplication (e.g., contamination of sterile dressings by falling dust containing bacteria; contamination of drinking water with sewage).

"Clinical infection" (microbial disease) - occurs when changes result in functional damage to the infected host. Such changes occur when the balance between host and microorganisms is disturbed:

- Large no. of microbes	Versus	- Small no. of microbes
- Increased virulence	Versus	- Attenuated virulence
- Susceptible host	Versus	- Resistant host

CLINICAL INFECTION	SUB-CLINICAL INFECTION	NO INFECTION
= Disease	= Inapparent/asymptomatic	= O.K.

Microorganisms capable of producing clinical or subclinical infection are classified based on terms such as pathogenicity: ability of a microorganism to produce disease; and virulence: relative capacity of a microorganism, within a group, to cause damage resulting in disease (in other words the degree of pathogenicity of a particular microorganism).

Opportunistic pathogens are microorganisms which rarely cause disease in healthy humans, but often do so in humans whose defense mechanisms have been compromised or breached by a burn or instrumentation.

PATHOGENESIS OF INFECTIOUS DISEASE

When pathogenic microorganisms enter the body, two opposing forces are set into action: the microorganism - striving to multiply and invade the tissues; and the host - striving to block the invasion of microorganisms and destroy them.

The capacity of a microorganism to initiate an infection and produce disease is dependent upon:

1. **Transmissibility** - the transfer of an effective challenge amount from a source to a host.

1.1 **Routes of Entry**

a) Inhalation
b) Ingestion
c) Break in protective barrier
d) Direct deposit

2. **Pathogenicity** - the capacity to inflict damage as a results of invasiveness (ability to overcome host defences and multiply); toxigenicity (production of toxins); and both invasiveness and toxinogenicity

2.1 **Invasiveness**

Microorganisms overcome their host's immediate defence mechanisms through their ability to:

- adhere and persist on body surfaces
- protect themselves against bactericidal substances present in body fluids
- avoid ingestion and destruction by phagocytes

The means by which bacteria adhere to, evade the defence mechanisms and invade the host include:

2.1.1 Surface structures (pili, fimbriae) - adhere to specific receptors present on body cell surfaces.

2.1.2 Capsules - usually polysaccharides, protect the microorganisms against leucocytes

Enzymes - that, although not toxic "per se", may contribute towards the virulence of the pathogen that elaborates them. Examples include

2.1.2.1 coagulase: enzyme accelerating the clotting of plasma; the clot formed around the focus of infection constitutes a barrier against leukocytes and body fluids.

2.1.2.2 hyaluronidase: an enzyme which hydrolyses the polymerized hyaluronic acid; the latter is part of the intercellular ground substance of mesodermal tissue. Hyaluronidases, also known as "spreading factors" help bacteria by liquefying the viscous polymer and facilitating the spread of fluids carrying the bacteria.

2.2 **Toxinogenicity**

Toxins are substances produced by bacteria that damage host tissues or upset systems vital to the host. There are two classes of toxins: exotoxins and endotoxins.

2.2.1 Exotoxins:
- proteins excreted by **living bacterial cells.**
- have specific affinities for host systems, e.g., diphtheria exo- toxin poisons cardiac muscle and nervous tissue; botulinic exotoxin → nervous tissue.
- active in very small concentrations, e.g., botulinum neurotoxin, one of the most toxic substances known, can kill a man in a dose of 0.0001 mg (0.1 μg).
- thermolabile.

2.2.2 Endotoxins: Toxic substances associated structurally with the bacterial cell and **liberated only when the cell disintegrates.** They are less specific and less potent than exotoxins, but may produce marked clinical effects:
- pyrexia (fever)
- malaise
- vasomotor disturbances → shock (e.g., septic shock in some septicaemias).
- thermostable (at 100°C).

BODY DEFENCES (Immunity)

1. **Non-specific: immunity (or natural, or innate)**

Skin
- mechanical barrier
- acid pH: sebaceous secretions and sweat → unsaturated fatty acids, bactericidal.
- lower temperature → suboptimal for some bacteria.

Mucous membranes - mechanical barrier

- cilia of respiratory tract eliminate particles larger than 5 microns (e.g., large bacteria-carrying dust particles).
- lysozymes (antibacterial substance e.g., in tears)
- pH - e.g., gastric juice pH 1-2, acid pH in vagina, urine.

Iron-binding proteins (e.g., transferrin, lactoferrin) which bind the iron necessary for bacterial growth.

Phagocytosis - polymorphonuclear white blood cells and monocytes as well as fixed macrophages in the tissues engulf and eventually destroy bacteria.

2. **Specific immunity (or acquired, or adaptive)**

Mechanisms aimed at particular infecting organisms are divided into two major systems:

2.1 Specific circulating antibodies in body fluids ("Humoral Immunity").

2.2 Cells trained to attack specific invading organisms ("Cell Mediated Immunity").

Both types of immunity are induced in the body as a result of encounters with the microorganisms against which they are directed. Both types are "specific", i.e., they are directed against one particular species of microorganism and not any other.

2.1. Humoral Immunity

Depends upon the presence of circulating antibodies which are modified serum globulins, physico-chemically tailored to react with particular chemical components of previously encountered invading organisms and produced only in response to these encounters.

Antibodies are produced by B lymphocytes. In order to produce antibodies B lymphocytes need antigen-presenting cells; the production is regulated (modulated) by T-helper and T-

suppressor cells.

Humoral immunity plays an important role in infections in which the pathogenic mechanism involves production of toxins or presence of a capsule as well as in some viral infections.

The chemical components which stimulate the production of antibodies are termed "Antigens".

2.1.1 Antigen (Ag)

- must be recognized by body as foreign (i.e., "non-self").
- introduced into animal body stimulates the production of antibodies and reacts specifically with those antibodies
- usually a foreign natural protein, glycoprotein, lipoprotein, polysaccharide
- molec. wt. must be at least 10,000 to trigger an immune response
- can be particulate or soluble
- bacterial cells contain a number of antigenic molecules: capsular substance, flagella, cell wall, etc. Viruses have polypeptide antigens

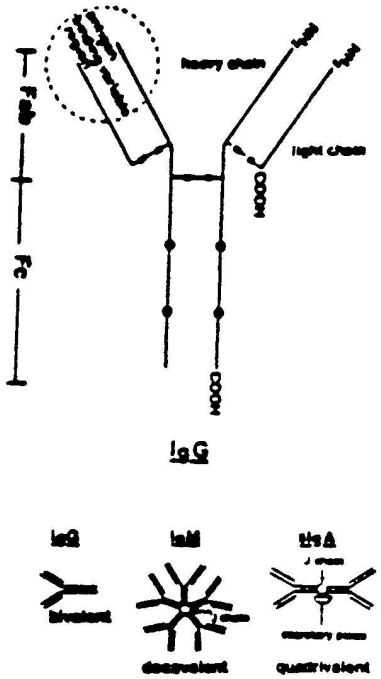
An antigen is a substance capable of inducing a specific immune response (e.g., antibodies)

2.1.2 Antibody (Ab)

- heterogeneous group of proteins called immune globulins (Ig)
- produced by the body in response to stimulation by antigens.
- antibodies are synthesized by B lymphocytes (plasma cells)
- antibodies have a remarkable ability to distinguish foreign macromolecules (NON-SELF) from 'normal' body constituents (SELF)
- exercise high specificity in combination with antigens

- Ab don't appear to differ significantly from each other in gross physical or chemical characteristics, but differ significantly in ability to combine with different antigens.

An <u>antibody</u> is an immune globulin produced in response to stimulation by an antigen and reacting <u>specifically</u> with it.



Immune Globulins are divided into five classes: IgG, IgA, IgM, IgE and IgD. IgG, IgA and IgM are involved in the defense mechanisms. IgE is involved in some hypersensitivity states. The role of IgD is not completely elucidated. The basic unit found in IgG is a Y-shaped molecule. Each Y-shaped IgG molecule has two combining sites which combine specifically with the antigens, like a key in a lock. The rest of the molecule can hook up onto phagocytes and macrophages, which eventually destroy the microorganism. It can cross the placenta and protects the newborn.

IgM is made of 5 units joined. IgM is the main immune globulin produced in the early immune response. It does not cross the placenta.

IgA is found in secretions (e.g., secretions on the mucosae of the respiratory, gastrointestinal and genito-urinary tract, tears, milk, etc). The secretory IgA molecule is made of two units joined together.

2.1.2.1 The Primary immune response

The first introduction of an antigen into the body triggers the production of antibodies against the foreign substance - the primary response:

- lag (latent) period for up to several days
- circulating antibodies detectable in 5 - 10 days
- serum antibody peaks at about 3 weeks then level drops (eventually to undetectable levels)

2.1.2.2 The secondary immune response

The secondary immune response occurs when an antigen is introduced for the second time, third time, etc. After a short lag, the titer of antibody rises rapidly (2-3 days) and then decreases over a much longer period, stabilizing at a lower level. Secondary response may be repeated several times until antibody level reaches a maximum usually after 3 - 5 injections of antigen. These are called booster injections.

The primary, secondary immune responses and booster injections constitute the basis of immunization (see section on immunization).

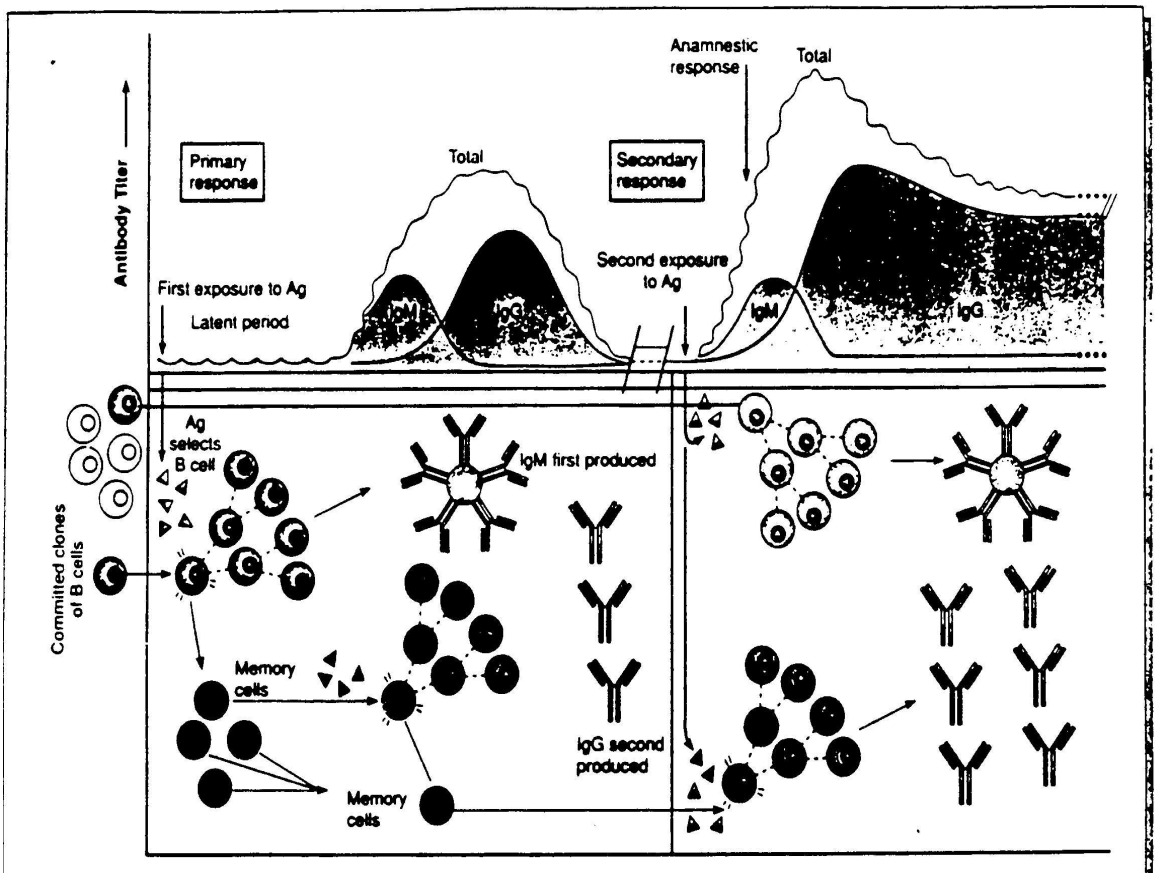


Figure: Primary and secondary responses to antigens

Primary and secondary responses to antigens. Top: The pattern of antibody titer and subclasses as monitored during initial and subsequent exposure to the same antigen. Bottom: A view of the B-cell responses that account for the pattern. Depicted are clonal selection, clonal expansion, production of memory cells, and the predominant Ab class occuring at first and second contact with Ag.

2.1.2.3 The serological reaction:

- used to demonstrate the presence of antibodies in a serum sample: eg screening of blood donors.
- usually results in an observable antigen-antibody reaction
- permits quantitation of antibody in patient sera i.e., titration of antibody.
- unknown microorganisms can be identified with known diagnostic antisera

2.2 Cell-Mediated Immunity (CMI)

The basic mechanism of CMI is similar to that of humoral immunity in that exposure to an antigen induces production of "trained" cells active against that antigen or any organism that carries it. The main difference is that soluble antibodies are not involved. Cell-mediated immunity is based on a large number of T-cell subpopulations and a complex system of interactions. Cell-mediated immunity is active in most microbial infections and is essential in the defense against intracellular organisms (including viruses) as well as in the defense against parasites, tumor cells and foreign cells (e.g., grafts, transplants).

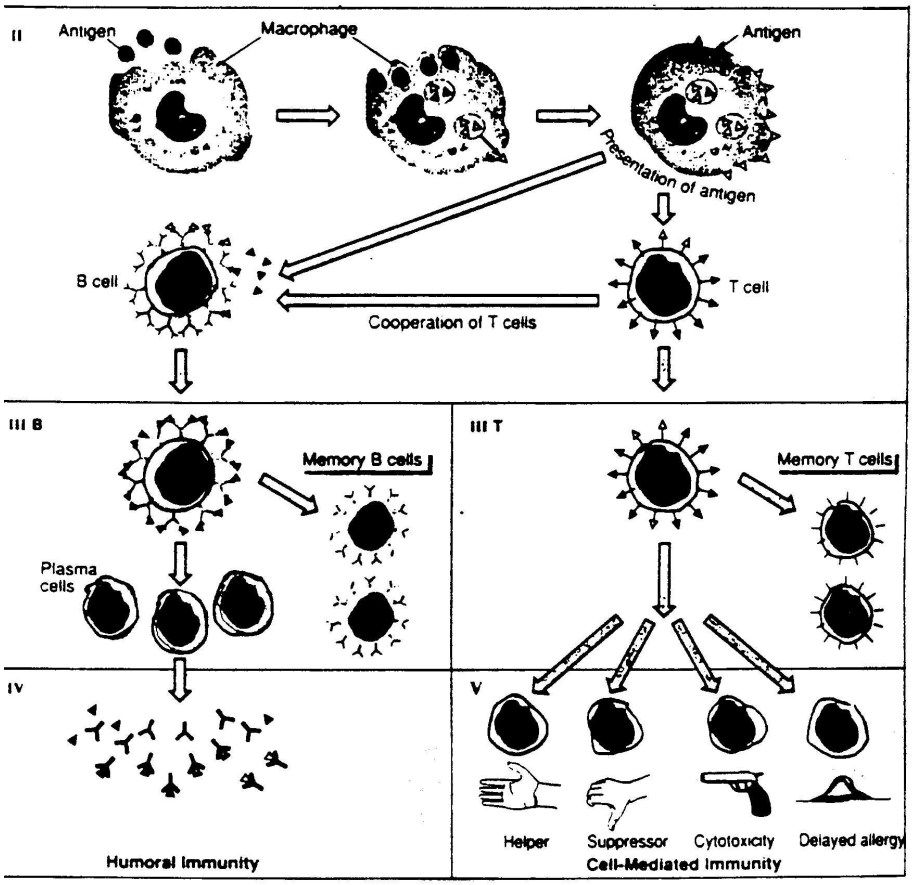


Figure: overview of the stages of lymphocyte functions

II: Antigen processing by macrophage and presentation to lymphocytes; assistance to B cells by T cells. IIIB and IIIT: Lymphocyte activation, clonal expansion and formation of memory B and T cells. IV: Humoral immunity. B-cell line produces antibodies to react with the original antigen. V: Cell-mediated immunity. Activated T cells perform various function on the original antigen.

2.3 <u>The immune system is not perfect!</u>

The immune system and the defense mechanisms are not perfect. Age, race, stress, nutritional status, etc affect the quality and the intensity of the immune system, and some individuals will fall ill and suffer from infectious diseases.

3. **Harmful Effects of the Immune Response = Disorders of Immunity**

There are 4 situations in which the immune system can be harmful. It is important to realize, however, that these are the exception.

3.1 Allergy and hypersensitivity states

In these situations, there is overreaction to antigens, in the absence of infection. These reactions can be explosive, with airway obstruction and circulatory collapse, also called **ANAPHYLAXIS**. Such reaction can be observed when administering vaccines to individuals who are allergic to a component of the vaccine, such as traces of egg proteins, etc.

3.2 Auto-immune diseases

The immune system of some individuals can react to its own material ("SELF"), including the formation of auto-antibodies.

3.3 Immunodeficiency states

Some individuals lack the ability of producing antibodies, cell-mediated immunity or both. This can be the result of congenital abnormalities, irradiation or disease, such as AIDS.

3.4 Graft rejection (kidney, bone-marrow, heart, etc)

In transplanted patients, the rejection of the graft is a normal reaction of the immune system, which recognizes the foreign grafted organ. But this is harmful to the patient and has to be controlled by drugs.

IMMUNIZATION (VACCINATION)

See Canadian Immunization Guide for general considerations, recommended immunization schedules for adults and children, types of active and passive immunization agents, immunization of health care workers, foreign travel, anaphylaxis management, etc. Information can be found at

http://www.phac-aspc.gc.ca/publicat/cig-gci/index-eng.php

Definitions:

A) Passive immunization

Administration of preformed antibody (=IgG) against a particular microbial agent, usually by the intramuscular route. The preformed antibody is obtained from humans or derived from animals, usually horses, which have been actively immunized against the particular microbial agent. The protection is immediate, but:

1. IgG of animal origin is recognized as foreign by the immune system and cleared from the recipient in about l0 days; the passive immunity is therefore short-lived. Besides, it carries the risk of hypersensitivity reactions (IgE antibodies) such as serum sickness and anaphylaxis.

2. IgG of human origin also disappears from the circulation after several weeks and the passive immunity is therefore also short-lived! However, it does not carry the risk of hypersensitivity reactions.

Immune serum globulin or gamma globulin is the IgG fraction pooled from a large group of blood-donors. It contains antibody to many naturally occurring diseases. Hyperimmune globulins are IgG fractions from human subjects with high titers of antibody to a specific disease that have resulted from

natural exposure or hyperimmunization.

B) Active immunization

Stimulation of the body's immune mechanisms through administration of a vaccine = an antigen = an immunogen from a microbial agent. Longer-lasting than passive immunization.

1. Live attenuated vaccines generally result in a subclinical or mild illness that duplicates, to a limited extent, the disease to be prevented. They usually provide both local (IgA) and humoral immunity (IgG), which develop more rapidly than with killed vaccines.

   Serious overt disease from the vaccine itself can result in patients with immunodeficiency syndrome such as AIDS or in patients whose immune responses have been suppressed.

2. Killed vaccines, subunits vaccines and toxoids are immunogens without infectivity. They must be administered parenterally with one or more spaced injections to give a satisfactory secondary response. They may also need subsequent boosters. Toxoids are usually administered in the presence of an adjuvant. Some polysaccharide vaccines are conjugated to a protein (conjugate vaccines).

3. Recombinant vaccines: these are produced by DNA recombinant technology, which avoids the possibility of a live virus surviving the inactivation process. The best known example is hepatitis B vaccine.

4. Adsorbed vaccines: vaccines mixed with inorganic salts (such as alum), to provide an injectable preparation that is more slowly adsorbed by the tissues, thus ensuring a prolonged immunogenic effect. Applies to tetanus and diphtheria toxoids.

5. Conjugate vaccines: vaccines such as *Hemophilus influenzae* type b, contain a

polysaccharide capsule that is poorly antigenic. Nevertheless, antibodies against this capsule protect against infection. In a conjugate vaccine, the capsular material is attached (conjugated) to an altered, non-toxic protein. In this form, the polysaccharide becomes immunogenic.

6. Combined vaccines: Several live attenuated vaccines or several killed vaccines, subunits vaccines and toxoids can be combined together to facilitate their administration.

7. Schedule of immunizations: this term applies to the orderly time-table under which several routine immunization procedures are carried out in infancy and childhood.

C) Combined active-passive immunization

This procedure is used for a few situations, in which, after a potential exposure to a given microbial agent, it is desirable to provide the patient with immediate protection through passive immunization while at the same time proceeding with active immunization: tetanos, hepatitis B, rabies exposures. The hyperimmune globulins and the vaccine are injected at two different sites, with different syringes.

ANTIBIOTIC RESISTANCE

1. History and significance of antibiotic resistance

Antibiotics such as penicillin, when introduced into clinical use in the 1940s and 1950s, were hailed as miracle drugs. Antibiotics significantly reduced the morbidity and mortality associated with pathogenic bacterial infections. In recent years, however, the efficacy of antibiotics has been dramatically reduced. Both the prevalence and incidence of antibiotic resistance is increasing in Canada, North America and throughout the world. The increases in antibiotic resistance severely limits the agents available to treat patients with serious infectious diseases. Antibiotic resistance reported for all the major antibiotic classes has resulted in higher morbidity, greater mortality and markedly increased hospitalization costs. Reasons underlying the marked increase in antibiotic resistant bacteria include:

(a) Use and misuse of antibiotics in agriculture and aquaculture.
(b) Use and misuse of antibiotics in the human population (mainly in developing countries).
(c) With advances in medical therapies, many more immunocompromised patients are remaining alive longer and are serving to harbour and transmit antibiotic resistant bacteria.

To add to the seriousness of the problem, the emergence of multiple antibiotic resistant (Mar) strains, which develop simultaneous resistance to most of the commonly used antibiotics, has rendered some infections untreatable. Therefore, there is an increased demand for the understanding of the mechanisms of different antibiotic resistance and for the pharmaceutical industry to develop new generations of antimicrobial agents.

2. Antibiotic Resistance Mechanisms

Bacteria will develop resistance to virtually any antibiotic given sufficient time. Resistance develops more often and more quickly in immunocompromised patients resulting in increased morbidity,

increased length of hospital stay and higher mortality. Antibiotic resistance is generally classified into two major types **intrinsic resistance** and **acquired resistance**.

(a) Intrinsic resistance - a predictable form of resistance based on the mechanism(s) of action of the antibiotic and the characteristics of the microorganisms.

(b) Acquired resistance - described as a previously susceptible organism becoming resistant to an antibiotic's action. This type of resistance is clinically more important and involves three main mechanisms of resistance:

(i) Alteration in drug target
(ii) Production of inactivating enzymes
(iii) Decreased antibiotic uptake

Table 1 Major Resistance Mechanisms to Antibiotics

Resistance Type/Antibiotic Affected	Mechanism of Resistance
Altered Target	
Aminoblycosides (streptomycin)	Altered ribosomal protein
β-lactams	Altered or new penicillin binding proteins
Clindamycin and Erythromycin	Ribosomal RNA methylation
Fluoroquinolones	Altered DNA gyrase
Rifampin	Altered RNA polymerase
Sulfonamides	New drug-resistant dihydropteroate synthase
Tetracycline	Ribosomal protection
Trimethoprim	New drug-insensitive dihydrofolate reductase
Vancomycin	Altered cell-wall stem peptide
Inactivating Enzymes	
Aminoglycosides (gentamycin)	Acetyl-, nucleotidyl- or phosphotransferase
β-lactams	β-lactamase
Chloramphenicol	Acetyl transferase
Decreased Uptake	
Decreased permeability	
β-lactams, chloramphenicol, tetracycline, fluoroquinolones, trimethoprim	Altered outer-membrane proteins
Efflux	
Erythromycin, fluoroquinolones, tetracycline	New membrane transport system

3. Genetics of Antibiotic Resistance

Antibiotic resistance genes can be encoded by the bacterial chromosome or by extrachromosomal entities such as plasmids. Antibiotic genes may confer resistance by :

(a) Exchange of genetic material

(i) Conjugation (plasmid) - This mode of exchange requires cell-to-cell contact as well as specialized bacterial structures known as conjugative pili for the transfer of DNA molecules usually in the form of plasmids, which are extrachromosomal DNA elements that replicate in bacteria. Plasmids containing genes that allow them to initiate their own transfer from organism to organism are called conjugative plasmids. Conjugation is a powerful and sophisticated mechanism of horizontal gene transfer, however, all plasmids have limited host ranges.

(ii) Transformation - This refers to the uptake of free or naked DNA from the environment, its incorporation into the bacterial genome and subsequent gene expression . Recent research data have shown that DNA transformation does occur in nature with a consequent increase in antibiotic resistance.

(iii) Transduction - This involves the transfer of genetic material among bacterial cells using a bacteriophage as vector or carrier. Transduction is an entirely random mechanism depending on the accidental incorporation of bacterial DNA within a phage particle. Due to limitations such as high host specificity and host restriction/modification systems, transduction has limited importance among clinically important bacteria. One exception is the evidence of transduction being an important mechanism of natural plasmid transfer among *Staphylococcus* spp.

(iv) Transposition - this is a powerful mechanism for mobilizing antibiotic resistance genes from one DNA molecule to another, such as from a chromosome to a plasmid.

Conjugative transposons can jump from the chromosome of one organism to the chromosome of another thus circumventing plasmid-host range restrictions. Another important group of genetic element, known as intregrons, is a mobile DNA element with a specific structure consisting of two conserved segments flanking a central region in which "cassettes" that encode functions such as antibiotic resistance may be inserted.

(b) Chromosomal alteration or activation

Antibiotic resistance may be encoded by bacterial chromosomes. The origin of the resistant phenotype is usually associated with mutation of a chromosomal gene involved with either:

(i) encoding the target site of the antibiotic, rendering the site functional but nonsusceptible.

(ii) being a regulatory element which controls alternative pathways or efflux mechanisms.

(iii) controlling cell permeability and regulating the uptake of the antibiotic and, consequently, the intracellular concentration of the drug.

A chromosomal locus, *mar*, discovered in the early 1980s, produces a phenotype known as **m**ultiple **a**ntibiotic **r**esistant (Mar). Evidence have shown that *mar*-related loci may be widely distributed among different bacteria. Therefore, activation of the *mar* locus, resulting in bacteria that have a Mar phenotype, may be a clinically important form of multiple drug resistance.

General Principles of Diagnostic Microbiology

Isolation of pure culture from specimen

Microorganisms in nature exist as mixed cultures and are therefore difficult to study. In order to characterize microorganisms, one must isolate different species from a specimen.

Nutrient material called culture media used to grow and isolate microorganisms

The type of media used based on many factors to be considered:

i. Source of sample tested
ii. Species suspected to be in sample
iii. Nutritional requirement of the suspected organisms

Small portion of sample (inoculum) is used to inoculate different media to isolate microorganisms

Methods of inoculation include:

i. Streak plate method
ii. Spread plate method
iii. Pour plate method

Inoculated media must then be incubated at the appropriate temperature (normally 37°C) to allow microorganisms to grow and multiply. When enough cells have divided (~a few million), colonies are formed which are visible to the naked eye. Each colony is derived from a single cell and all cells within a colony are identical to each other. Different species have different colonial morphologies therefore can be used as mean of distinguishing different species in a mix culture.

Preservation of pure cultures

Short term preservation - cultures can be stored in medium refrigeration temperatures (4 to 10ºC).

Long term preservation :

i. Frozen in liquid nitrogen (-196ºC)
ii. Frozen in special freezer (-70ºC to -120ºC)
iii. Lyophilization (freeze drying) - dehydration followed by vacuum sealing (most stable form of storage)

Identification of microorganism after obtaining as pure culture

Two approaches to the study of microorganisms after being isolated as a pure culture include the colonial morphology and the cellular morphology. The latter requires the use of microscope.

The size of most microbial cells and viruses is in range of nanometer to micrometer and so there is a need for magnification by using a microscope. A microscope's useful magnification limited by its resolving power. Resolution is the ability to distinguish two closely located objects as separate, distinct entities. Resolution is fixed by the wavelength of light used and by the optical properties of the lenses.

Light microscope - system of lenses used to manipulate the path a light beam travels between the specimen and the eye.

Electron microscope - a beam of electrons controlled by a system of magnetic fields used in place of the light source of a light microscope.

Staining Techniques

The basic protocol for staining of microorganisms is as follows:

a. A thin film of specimen (smear) is place onto a clean microscope slide and let air dry.
b. The dried smear is fixed by heat to make microorganisms stick to glass slide.
c. Stain with one or more dyes prior to viewing with microscope.

Simple staining vs. differential staining

Simple staining refers to staining by a single general dye colors all microorganisms in a specimen and allows the observation of size, shape, number and arrangement of cells. An example would be methylene Blue staining. **Differential staining** occurs when two or more special dyes are used to observe differences between microbial cells or parts of cells. Examples of differential staining include the following:

- acid fast stain - carbolfuchsin and methylene blue used to differentiate acid-fast bacteria such as *Mycobacterium* from other non-acid-fast bacteria.
- Gram stain - characterize bacteria into two groups: gram positive and gram negative.

The Gram stain is perhaps the most important staining procedure in microbiology!

Cell Wall

- rigid structure giving characteristic shape of bacterial cell.
- essential for cell growth and division.
- Gram negative cell wall usually thinner than gram positive cell wall.
- in eubacteria shape determining part is rigid material called peptidoglycan.
- archaeobacteria do not have peptidoglycan.

(i) Gram positive cell wall

- thick structure made up mainly of thick layer of peptidoglycan
- teichoic acids often found attached to peptidoglycan to give negative charge to help transport of positive ions and storage of phosphorus.

(ii) Gram negative cell wall

- more complex
- has a outer membrane covering a thin layer of peptidoglycan (i.e., in periplasmic space between outer and cytoplasmic membranes)

- outer membrane anchored to peptidoglycan by a lipoprotein
- another structure found only in outer membrane is lipopolysaccharide (LPS)
- outer membrane is a selective barrier based on size and charge of molecules.
- in gram strain, alcohol increases permeability of gram negative outer membrane but shrinks pores of gram positive peptidoglycan.

Difference in staining is determined by different cell wall structures.

Other types of staining techniques include:
a. Endospore staining - malachite green applied with heat to penetrate spores followed by counter -staining with safranin.
b. Capsule staining - treat with copper sulfate before staining to visualize capsule as a clear zone surroundings cells.
c. Flagella staining - use of mordant to thicken flagella before staining to visualize.

Fluorescence Microscopy is a useful tool when trying to reveal only objects that are of interest in an otherwise black background. In order to do this, a special dye which fluoresces at a specific wavelength is visualized using a light microscope equipped with the appropriate filters. Immunofluorescence is a common and important aspect of fluorescence microscopy. For example, an antibody is produced in blood after exposure to foreign material such as bacteria. This antibody reacts (binds) to the foreign particles forming a complex. In an immunofluorescence assay, a fluorescent dye is attached to a specific antibody and this complex is added to a specimen. Any attachment of the labelled antibody to microorganisms in the specimen will fluoresce under the appropriate conditions and can be easily detected.

Electron Microscopy
The short wavelength of the electron beam as compared to light allows for greater resolving power (0.003 um). Magnification of 1 million times is possible. There are two common electron microscopy approaches that are used. Transmission electron microscopy requires staining with heavy metals of

whole specimen or slicing of microorganisms into thin sections; whereas in scanning electron microscopy, an electron beam moving back and forth generates a 3-dimensional image of cell surface of microorganisms coated with a fine film of metal.

*Importance of studying detailed morphology of microorganisms include

a. absence or presence and characteristics of cellular structures help classification
b. morphology of cells help them to respond to environment.
   (e.g., extracellular structures can make microorganism more pathogenic)

Bacteria

Bacteria are small (0.75 to 1.25 um in diameter/width) but have a much higher ratio of surface area to volume than larger, more complex organisms (i.e., needed for more efficient means of nutrient entry which is partly responsible for high rate of metabolism, growth and replication. This is one major reason why bacteria are heavily used in molecular biology studies. One of the most common, a non-pathogenic strain of *Escherichia coli*, has a division time of ~ 20 min.

Bacterial vary in shapes. All have one of three basic shapes:

a. Spherical cells (called coccus) - usually round, sometimes ovoid
b. Cylindrical or rod shape (called bacillus) - vary in width and length, ends can be square, rounded, tapered or pointed.
c. Spiral or helical shape (spirillum) - corkscrew like shape

* Not all bacteria have exactly these 3 general shapes. A few can change cell shape as it grows. These are called pleiomorphic organisms.

Individual bacterial cells often arranged in specific patterns useful for identification:

a. spiral-shaped and rod-shaped bacteria usually exists as single cells
b. when coccus divide in one plane = diplococcus (pairs) e.g., *Neisseria gonorrheae*
c. when coccus divide in one plane but remain attached to form chain = streptococcal

arrangement, e.g., *Streptococcus* spp.

d. when coccus divide at right angle to the first plane of division = tetrads, e.g., *Pediococcus*

e. a further division in third plane = cubical packet of 8 cells called sarcinae, e.g., *Sarcina* spp.

f. division in 3 planes in an irregular pattern = grapelike clusters, e.g., *Staphylococcus*

* Not all bacteria are found in their perfect arrangement under microscope. It is useful to look for predominant patterns of arrangement.

* Size, shape and arrangement of bacterial cells is referred to as gross morphology.

In order to isolate a microorganism, specific media is required:

1- Chemically defined media. - Exact composition of media know thus can alter individual component separately.

Undefined media - natural products (e.g., blood, beef extract, peptone, etc.) added to media for routine lab cultivation.

2- When solid support needed, 1.5 % agar is used.

3- Media for growing bacteria - requirements varies between different bacteria.

Microorganisms with demanding nutritional requirements are known as fastidious. They require complex, undefined media for cultivation.

4- Media for growing yeasts - all fungi and heterotrophs (requires organic substrates to get its carbon source for growth and development).

- generally have higher sugar content and lower pH than bacterial media.

5- Media for anaerobes:

-anaerobes are those that tolerate little or no oxygen (sometimes grown deep down in agar or put media in tall test tubes.

6- Selective media - media designed to enhance growth of one kind of microorganism or suppress growth of other kind of microorganism or both.

For example, brilliant green agar is used to isolate *Salmonella* species from food samples. That is, the brilliant green agar inhibits Gram-positive bacteria. Another example is phenylethanol agar that inhibits Gram-negative bacteria.

7- Differential media - used to differentiate organisms based on their unusual nutritional requirements and characteristic appearance in media (e.g., lysis of red blood cells, hemolytic vs. nonhemolytic bacteria).

8- Selective/Differential media - useful in public health microbiology - e.g., MacConkey medium (bile salt and crystal violet inhibits gram-positive bacteria thus allowing Gram-negative organisms).

9- Enrichment media - used to increase number of specific organism in a sample by favoring the growth of the interested species.

10-Tissue culture media - plant or animal cells grown in lab in specialized media used to cultivate viruses in vitro since viruses can only replicate inside living host cells.

Four main conditions make up physical conditions essential for successful cultivation of microorganisms:

A. Temperature

- Microorganisms in general can grow over a wider temperature range than more complex organisms (e.g., *Bacillus subitlis* can grow from 8 to 53ºC).
- Temperature at which a species grows most rapidly is the optimum growth temperature.
- Cardinal temperatures of a species of microorganisms
    (i) Minimum temperature
    (ii) Optimum temperature
    (iii) Maximum temperature
- Cardinal temperatures changes depending on nutritional content of growth medium.
- Optimum temperature usually closer to maximum temperature since enzyme activity increases with temperature until upper limit at which enzymes is degraded is reached.
- Microorganisms are divided into 3 major groups based on their optimum growth temperature:
    (i) Psychrophiles - grow best from 15 to 20ºC (may die if exposed to room temperature for short time probably due to damage to cytoplasmic membrane). Bacteria and fungi from this group found in colder waters and soils such as oceans and the polar regions. Particularly problem of food spoilage, e.g., psychrophilic bacteria are Pseudomonas and Flavobacterium.
    (ii) Mesophiles - majority of microorganisms belong to this group. Grow best between 25 to 40ºC. Saprophytic organisms grow at lower part of the mesophilic temperature range. Parasitic organisms of humans and animals grow at upper part of this range (~37ºC).
    (iii) Thermophiles - can grow from 40 to 85ºC but grow best between 50 to 60ºC. Mostly procaryotes (eucaryotes cannot grow above 60ºC). Commonly found in volcanic areas, compost heaps and hot springs. E.g.,*Bacillus stearothermophilus.* Enzymes are rapidly produced to replace those damaged by high temperatures.

B. Gaseous Atmosphere

- Right combination of gases such as oxygen, carbon dioxide, nitrogen and methane resembling that found in natural habitat essential for cultivation of microbes in vitro.

(i) Aerobic Microorganisms - includes microbes which can grow in standard atmosphere of 21% oxygen. E.g., *Mycobacterium*, *Legionella,* filamentous molds. Require more energy than those microorganisms. No problem with oxygen supply when grown on surface of plates. In broth, require higher levels of carbon dioxide. E.g.,*Neisseria gonorrhoeae* (5 - 10 % carbon dioxide). Can use candle jar or more complex gas jars.

(ii) Anaerobic Microorganisms - may be poisoned by oxygen, cannot grow in air atmosphere, do not use oxygen for energy-yielding chemical reactions. Wide range in oxygen tolerance. E.g., *Clostridium perfringens* (highly oxygen-tolerant); *Clostridium tetani* (Moderately tolerant); and *Methanobacterium*, *Methanospirillum* (strict anaerobes, i.e., killed by brief exposure to oxygen). Toxicity of oxygen due to production of superoxide radical, hydrogen peroxide and hydroxyl radicals. Aerobes protect against these radicals by producing enzymes such as superoxide dismutase, catalase and peroxidase. Anaerobic jars, anaerobic chamber or anaerobic glove box can be used cultivate anaerobes.

(iii) Facultative Microorganisms - grow in air atmosphere but can also grow anaerobically. Do not need oxygen but can use it for chemical reactions. E.g., E.coli and Saccharomyces cerevisiae (common baker's yeasts).

(iv) Microaerophilic Microorganisms - can use oxygen for chemical reactions. Grow best between 1 to 15% oxygen level. E.g., *Campylobacer jejuni*.

C. pH

- optimal pH is different for various organisms.
- regardless of the external pH, microorganisms must maintain intracellular pH at ~7.5 (this is done by the ability of the cell to expell or uptake hydrogen ions.

- most bacteria can grow at minimum pH of 4 and maximum pH of 9. Optimum pH normally lies between 6 and 8.
- molds and yeasts generally have a broader pH range for growth than bacteria. Optimum pH is ~ 5 to 6.
- growing cells release acidic or alkaline waste products into the growth medium environment. Without buffering of the medium, the shift can eventually inhibit growth.

D. Other conditions

- water, and sometimes light are important for certain microorganisms.
- osmotic pressure is another consideration. In a hypertonic solution (i.e., higher solute concentration in environment than cell cytoplasm, the cell loses water and eventually shrivel up). In a hypotonic solution, lower concentration of solutes in the environment leads to inflow of water resulting cell rupture. In an isotonic solution, no net flow of water occurs, resulting in normal cell growth.

GRAM POSITIVE COCCI

A. STAPHYLOCOCCI

Gram-positive cocci (l µm diameter) growing in grape-like clusters. Species particularly important for humans include:

Staphylococcus epidermidis - normal flora of skin and mucosae; occasionally pathogenic

Staphylococcus aureus - pathogenic; often found as normal flora

Staphylococcus saprophyticus - in the environment and on skin; can cause urinary infections

Staphylococcus aureus

Grows well on simple media (agar, blood-agar) producing 2-3 mm colonies in 24 h (37ºC).

Toxins: Cytotoxins (α, β, δ, γ, P-V leukocidin)

- toxic to many cells: leukocytes, erythrocytes, macrophages, platelets and fibroblasts

Haemolysins - dermonecrotic, lethal

Enterotoxin (A-E, G-I)

- superantigens
- important cause of food poisoning (nausea, vomiting, cramps, diarrhoeas)

Exfoliative toxins (ETA, ETB)

- "scalded skin" syndrome in infants

"Toxic Shock Syndrome Toxin l" (formerly pyrogenic exotoxin C and enterotoxin F)

- cause of toxic shock syndrome.

Enzymes: Coagulase - produced by almost all pathogenic staphylococci; coagulates fibrin.

N.B. *In vitro* the coagulase test is used as the main test for the identification of *Staphylococcus aureus.*

Beta-lactamase (penicillinase) - destroys penicillin. Other enzymes are also produced, e.g., hyaluronidase, staphylokinase, etc.

Many of the *S. aureus* strains in the normal population and 85-90% of strains isolated in the hospital are penicillin resistant.

Staphylococcus aureus is widely spread in the normal population; up to 15% of healthy individuals may carry it at one time or another in the anterior nares, axilla, perineum, etc. and on hands.

Clinical findings - *S. aureus* tends to produce localized purulent infections (pustules, boils, styes, conjunctivitis, otitis, etc). Complications which are generally very serious include pneumonia, osteomyelitis, septicaemia, endocarditis and others, particularly in compromised/immunosuppressed hosts (diabetics, leucaemia/lymphoma, steroid therapy, etc).

Other diseases caused by staphylococci include food poisoning, toxic shock syndrome, scalded skin syndrome, etc.

Staphylococcus aureus is an important cause of hospital acquired nosocomial infections, from stitch abscesses to extensively infected surgical wounds or generalized infections often with strains resistant to the commonly used antibiotics.

Epidemiology - *S. aureus* strains are lysed by a number of different bacterial viruses (bacteriophages). Cells originating from the same parental cell are lysed by the same bacteriophage. This makes possible the use of "bacteriophage types" as identity markers in tracing sources of infection.

Preventive measures against staphylococcal nosocomial infections include:

- Strict adherence to aseptic techniques in the operating rooms and to wound precaution techniques in handling post-operative infections

- Education of hospital personnel in the mode of transmission (mainly by contact) of staphylococcal infections and in the paramount importance of handwashing.

Staphylococcus epidermidis

Part of normal skin/mucous membranes flora, non-pathogenic. In a small number of compromised patients, *Staphylococcus epidermidis* and other coagulase-negative staphylococci can cause infections such as post-operative infections after brain or open-heart surgery, endocarditis after prosthetic heart valve insertion, shunt infections, etc.

In neonates may be the cause of necrotising enterocolitis.

B. STREPTOCOCCI

Gram-positive cocci usually arranged in pairs or forming chains. Fastidious in their nutritional requirements (grown on blood agar), and are subdivided according to:

a)Haemolytic properties: alpha-haemolysis, greenish brown zone of partial red blood cell destruction
beta-haemolysis, clear zone of complete red blood cell destruction around colonies
some strains are non-hemolytic

b) Carbohydrate C antigen: extracted from the cell wall, subdivides streptococci in groups A-T (Lancefield classification).

c) M-protein: Permits subdivision of beta-haemolytic streptococci into over 70 serotypes; found almost exclusively in group A. Important virulence factor: antiphagocytic and degrades complement C3b

Streptococcus pyogenes

Group A, beta haemolytic, and is the cause of:

Acute tonsillitis - ("strep sore throat")

- complication can result in Scarlet fever

Skin infections - cellulitis, erysipelas, wound/burn infections.

Puerperal fever (sepsis) - post-partum or post-abortion

Septicaemia

Complications following streptococcal infections, particularly in young patients:

(l) acute glomerulonephritis; and

(2) rheumatic fever involving joints and heart valves. *S. pyogenes* produces toxins and enzymes related to virulence.

Toxins: Streptolysins (O and S), beta-haemolytic and highly toxic for neutrophils and macrophages. An anti-streptolysin-O (ASO) antibody reaches high titers after recent infections (EXCEPT in skin infections).

Streptococcal Pyrogenic Exotoxins (Spes)

- superantigens SpeA, SpeB, SpeC
- causes the rash in scarlet fever

Enzyme: Hyaluronidase - splits hyaluronic acid in connective tissue and helps spreading.

Streptococcus pyogenes can be found in 5-l0% of healthy individuals. Practically all strains are sensitive to penicillin G which is the antibiotic of choice. Transmission by direct contact; nasal carriers are particularly likely to transmit the infection. Contaminated food may cause outbreaks.

Prevention includes:

- Education of health personnel and public on modes of transmission
- Strict asepsis in obstetric procedures
- Early detection and treatment (non-infectious within 24 hours)

STREPTOCOCCUS GROUP B (*Streptococcus agalactiae*) - frequently found in the vagina of healthy women, can cause neonatal infections:

1. Early septicaemia - respiratory distress/shock at birth or within 24 hours; high fatality rate
2. Delayed meningitic form - between l-l2 weeks post-partum; prognosis considerably better, but neurologic/mental abnormalities may result.

STREPTOCOCCUS GROUP D (*Streptococcus faecalis*, Enterococcus) - part of normal flora of the human gastro-intestinal tract. May cause infections when introduced into tissues: urinary tract infections, septicaemia, endocarditis, meningitis. Often resistant to a wide range of antibiotics.

VIRIDANS STREPTOCOCCI- includes a group of different species of streptococci (α-hemolytic and non-hemolytic) found in the oral cavity (mouth) of healthy individuals. May cause endocarditis in individuals with previously damaged heart valves.

Streptococcus pneumoniae (Pneumococcus) - diplococci (pairs) with a polysaccharide capsule having antiphagocytic properties. There are 90 distinct capsular serotypes. Pneumococci, which are commonly found in the naso-pharynx of healthy individuals, can cause:

Lobar pneumonia - most often an auto-infection, though it may spread as a "carrier epidemic". More frequent in infancy, old age and alcoholics

Meningitis more often in infants and in the elderly; accompanied by bacteraemia and eventually septicaemia

Prevention includes (l) programs for the elderly and alcoholics (2) avoidance of crowded living quarters and (3) vaccination of those at highest risk with a polyvalent pneumococcal vaccine containing 23 capsular polysaccharide serotypes.

GRAM NEGATIVE COCCI

Neisseria meningitidis (Meningococcus)

Found frequently in the naso-pharynx of healthy individuals; the "carrier state" may last from a few days to several months. Microscopically, Gram-negative cocci in pairs (diplococci).

Grow best on enriched media e.g., heated blood-agar ("chocolate agar") in 5-l0% CO_2. For isolation from naso-pharynx, selective media (e.g., Thayer-Martin) are used.

Infection with *Neisseria meningitidis* usually results in a carrier state and finally in immunization.

A few carriers develop generalized infection resulting in:

1. Meningitis - purulent, with high fatality rate if not treated promptly.

2. Septicaemia (meningococcaemia) - high fever, rash (from a few petechiae to haemorrhagic rash) and endotoxic shock.

3. Waterhouse-Fridrichsen syndrome - bilateral adrenal cortical haemorrhage with fulminating collapse/death in less than 24 hours. It is a complication of of meningococcal septiceaemea

There are 13 serogroups divided based on their polysaccharide capsule; most important A,B,C, X, Y and W135.

The capsular polysaccharides are anti-phagocytic. The disease is mainly a disease of children, but also a major problem in military recruits. Occasionally, can be epidemic.

Prevention and Treatment

- Conjugated vaccine that protects against serogroups A, C, Y and W135
- Vaccination is recommended for children 11-12 years, teenagers entering high school and college freshman residing in dormatories.
- Penicillin is the primary antibiotic used to treat infections; chloramphenicol or 3 rd generation cephalosporins can also be used.

Neisseria gonorrhoeae

The most common infections in humans are:

Genital infections in men (gonorrhoea) - acute infection of the urethra (purulent urethritis). Untreated may case epididymitis, prostatitis, etc.

Genital infections in women - usually asymptomatic (~50%) cervicitis, often infecting also the urethra and rectum. Untreated may lead to pelvic inflammatory disease (PID) i.e., salpingitis, pelvic peritonitis, adnexal abscesses, etc. often resulting in sterility. In both sexes, rectal gonorrhoea and/or pharyngeal infection are occasionally present.

Disseminated gonococcal infection (DGI) - gonococcal bacteraemia (in about l-3% of infected patients- usually women) with low grade fever, cutaneous-infection and arthritis in the wrists, knees and ankles.

Neonatal infections - gonococcal ophthalmia neonatorum (acute purulent conjunctivitis) rare but still occurring.

Laboratory diagnosis - *N. gonorrhoeae* grows well only on special media. Very sensitive to extremes of temperature and to drying.

Microscopy

Smears of urethral discharge in men showing intracellular, Gram-negative diplococci are for all practical purposes diagnostic (98% correlation with culture)

Culture
- mandatory for diagnosis of gonorrhoea in women (endocervical, urethral and anal swabs). Isolation from vaginal swabs is significantly lower
- Urethral swabs in heterosexual males with doubtful smears
- Urethral, anal and pharyngeal swabs in homosexual males.

Plates, Thayer-Martin or N.Y.C. (for New York City) agars should ideally be inoculate immediately and kept in an atmosphere containing CO_2 (e.g., candle jar). Different transport media have been devised (e.g., Stuart, Amies).

Epidemiology: In men, most infections are acute (about 90-95%) while in women 50% or more are asymptomatic. Over the years there has been a steady (but low level) increase in resistance to penicillin (chromosomal resistance). Strains highly resistant to penicillin (penicillinase producing *N. gonorrhoeae* or PPNG) have become prevalent in South-East Asia and West-Africa; also found in the U.S. and Canada. These highly resistant strains (plasmid-mediated resistance) have been selected through abusive use of penicillin.

Recommened treatment by the US CDC is ceftriaxone, cefixime, ciprofloxacin or ofloxacin in combination with doxycycline or azithromycin.

As in all sexually transmitted diseases, simultaneous treatment of both partners is essential in order to avoid reinfection.

GRAM-POSITIVE BACILLI

I. Spore-forming rods

There are basically 6 medically important Gram-positive bacteria

- 2 are cocci (staphylococci and streptococci)
- 4 are rods (2 spore-formers and 2 non-spore-formers)

The spore-forming, Gram-positive rods include *Bacillus* and *Clostridium*. They cause disease by the release of potent exotoxins. They differ biochemically by the ability (or lack thereof) to grow in the presence of oxygen. *Bacillus* likes oxygen (aerobic); whereas *Clostridium* grows in the absence of oxygen (anaerobic)

Bacillus species

There are 2 pathogenic species, *Bacillus anthracis* and *Bacillus cereus*. *B. anthracis* causes the disease anthrax while *B. cereus* causes gastroenteritis.

Bacillus anthracis

- unique…only bacteria with capsule made of protein (poly-D-glutamic acid)
- capsule prevents phagocytosis
- anthrax is a disease that primarily affects herbivores such as cows and sheep
- humans usually exposed to spores during direct contact with infected animals and/or soil
- spores of *B. anthracis* very stable, resistant to drying, heat, ultraviolet light, disinfectants
- spores germinate and makes toxins
- germination and expression of plasmid encoded virulence factors (on plasmids pXO1 and pXO2) is regulated by increase in temperature to 37ºC, carbon dioxide increases, and serum protein [Spores activate only when introduced into a host!!!]
- used for biological terrorism and warfare (used by Japanese army in Manchuria in 1940)

B. anthracis exotoxin:

- encoded on plasmid called pXO1
- exotoxin contains 3 separate proteins which are not toxic by themselves
- anthrax:
    - edema factor (EF): disrupts water homeostatis
    - protective antigen (PA): promotes entry of EF into phagocytic cells (similar to B subunit of A-B toxins)
    - lethal factor (LF): zinc metalloprotease that inactivate protein kinase

- plasmid pXO2
    - encodes three genes require for synthesis of poly-glutamyl capsule

Note:

- both plasmids are needed for bacterial virulence!!!!
- rapid identification and prompt use of penicillin, doxycyclin, ciprofloxacin or levofloxacin are critical in prevention of high mortality
- vaccine against PA available for humans; animals sometimes vaccinated with live cultures that have had their capsules removed

Bacillus cereus

B. cereus is different from *B. anthracis* in that is it motile, non-encapsulated and resistant to penicillin. It causes food poisoning (nausea, vomiting and diarrhea) when spores are present in foods, which then survive the initial cooking process. The bacteria then germinate in the food and release the enterotoxin. [Cooked foods must be exposed to high temperature and/or refrigeration in order to inactivate the spores!]

- 2 types of enterotoxins are made
    i. Heat-labile toxin (similar to cholera and LT from *E. coli*)
    ii. Nausea, abdominal pain, diarrhea
    iii. Lasts usually 12-24 hours

- Heat-stabile toxin
    i. Similar to *S. aureus* food poisoning
    ii. Short incubation period
    iii. Severe nausea and vomiting
    iv. Limited diarrhea

If patient presents with food poisoning (and examination of food reveals *B. cereus*) antibiotic therapy will not alter the course of patient symptoms. This is because the pre-formed toxins are what causes the food poisoning!

Clostridium species

Clostridium are Gram-positive, spore-forming rods, but are anaerobic. Anaerobic culture helps to differentiate them from the spore-forming rods (i.e., *Bacillus*). *Clostridium* are famous for causing botulism, tetanus, gas gangrene and pseudomembranous colitis. Exotoxins are extremely powerful; rapidly diagnose or else your patient dies!

Clostridium botulinum

- produces lethal neurotoxin that causes rapidly fatal food poisoning
- neurotoxin blocks release of acetylcholine (Ach) from nerve terminals in autonomic nervous system
- flaccid muscle paralysis results
    i. afebrile
    ii. bilateral cranial nerve palsies
    iii. double vision
    iv. difficulty swallowing
    v. general muscle weakness (leads to sudden respiratory paralysis and death)
- adult botulism can result from eating smoked fish or improperly prepared home-canned vegetables

- proper cooking helps to destroy spores
- improper cooking (canning) results in anaerobic conditions that allow for growth and synthesis of neurotoxin
- infant botulism associated to fresh honey contamination with spores
    i. spores germinate and bacteria colonize infant intestinal tract
    ii. neurotoxin released
    iii. constipation for 2-3 days, followed by difficulty swallowing and muscle weakness ("floppy baby")

Treatment: antitoxin, which neutralizes the unbound free neurotoxin in the bloodstream; and intubation and ventilatory support until respiratory muscles resume activity.

Clostridium tetani

- classic puncture wound by a rusty nail but can follow skin trauma by any object contaminated with spores
- spores found in soil and animal feces
- once in wound, can germinate as long as there is a localized anaerobic environment
- exotoxin is called tetanospasmin, and causes sustained contraction of skeletal muscles (tetany)
    i. tetanus toxin taken up at neuromuscular junction and transported to central nervous system
    ii. toxin acts on the inhibitory Renshaw cell interneurons (prevents release of GABA and glycine, which are inhibitory neurotransmitters)
    iii. this inhibition of inhibitory interneurons allows motor neurons to send a high frequency of impulses to muscle cells (sustained tetany ensues)
    iv. clinical presentation of tetanus includes severe muscle spasms (trismus, aka, lockjaw), grotesque grinning expression (risus sardonicus)
    v. mortality is high once stage of lockjaw achieved
    vi. formalin-inactivated toxin (tetanus toxoid) given every 10 years as

booster and is part of the DPT (diphtheria-pertussis-tetanus) shot

Clostridium perfringens

- causative agent of gas gangrene
- spores mature in anaerobic conditions and produce gas
- devastated soldiers wounded in battle
- clinically, 2 classes of infection with *C. perfringens*:
- celulitis/wound infection
    i. necrotic skin exposed to bacteria
    ii. bacteria grows and damages local tissues
    iii. palpitation reveals moist, spongy, crackling consistency to skin due to pockets of gas (crepitus)
- clostridial myonecrosis
    i. when inoculated with trauma into muscles, secretes exotoxins that destroy adjacent muscle
    ii. results in gas formation when carbohydrates are fermented from action of enzymes
    iii. CT scan will show pockets of gas within muscles and subcutaneous tissue
    iv. As enzymes degrade muscle, get thin, blackish fluid exuding from skin
    v. fatal unless identified and treated very early with hyperbaric oxygen and antibiotics (such as penicillin), with removal of necrotic tissue

Clostridium difficile

- more commonly seen in hospitals than anthrax, tetanus or botulism
- responsible for antibiotic-associated pseudomembranous enterocolitis
- arises from overuse of broad spectrum antibiotics (ampicillin, clindamycin, and cephalosporins), which destroy normal intestinal flora
- *C. difficile* superinfects the colon, where it can release exotoxins
- Toxin A causes diarrhea

- Toxin B is cytotoxic to colonic cells
- Symptoms include severe diarrhea, abdominal cramping and fever
- As the name suggests, it is very difficult (French equivalent is "difficile" ☺) to give patients antibiotics)
- *C. difficile* is considered possible cause when patients develop diarrhea while on antibiotics (toxin in stool confirms diagnosis)
- Treatment includes the following
    i. Discontinue initial antibiotic regimen patient was on
    ii. Administer metronidazole or vancomycin by mouth since they are not absorbed orally into bloodstream.

II. Non-spore-forming rods

The two medically important non-spore-former, Gram-positive rods, include *Listeria monocytogenes* and *Corynebacterium diphtheriae*.

Listeria monocytogenes

- one of few bacteria that can cross the 3 protective barriers (blood-brain, gastrointestinal, and feto-placental)
- psychrophile
- can cause variety of symptoms, ranging from general malaise, to meningitis to spontaneous abortions (stillbirth) to death
- is considered a facultative intracellular organism (can live outside or within cells)
- immunosuppression (young, elderly, AIDS, transplant, etc.) = high risk individuals for disease known as listeriosis
- antimicrobial resistance, oddly enough, is not an issue with *L. monocytogenes* at the moment
- treatment consists of ampicillin or trimethoprim-sulfamethoxazole

Corynebacterium diphtheriae

- pathogen responsible for diphtheria
- colonizes the pharynx, forming a grayish pseudomembrane composed of fibrin, leukocytes, necrotic epithelial cells and *C. diphtheriae* cells
- from here, bacteria releases powerful exotoxin into bloodstream
- exotoxin damages heart and neural cells by interfering with protein synthesis
- treatment consists of 3 steps
    - antitoxin to inactivate circulating toxin
    - penicillin or erythromycin to kill the bacteria
    - DPT vaccine (D = diphtheria)

- *C. diphtheriae* must be lysogenized by a temperate bacteriophage

- Bacteriophage codes for diphtheria exotoxin, which contains two subunits
    - B subunit binds to target cells and allows A subunit to enter
    - A subunit blocks protein synthesis (inactivates elongation factor EF2)

GRAM-NEGATIVE BACILLI

The enterics are Gram-negative bacilli part of the normal flora found in the intestine. They also cause gastrointestinal disease. The major groups are Enterobacteriaceae, Vibrionaceae, Pseudomonadaceae and Bacterioidaceae. These organisms are divided into groups based on their biochemical and antigenic properties.

1. ENTEROBACTERIACEAE

This large family of closely related bacteria includes species associated with the intestinal tract of man and animals both as normal flora (*Escherichia coli*) and specific pathogens (*Salmonella*, *Shigella*) as well as soil bacteria and plant pathogens. Most species have the capacity to cause opportunistic infections in man.

1.1 SALMONELLAE

General Properties:

Lactose non-fermenters; motile. With the exception of *S. enterica* serovar Typhi, all have animal reservoirs. The nomenclature has changed over the years and the current one is as follows:

The genus *Salmonella* consists of two species, each containing multiple serovars. The two species are *S. enterica* and *S. bongori*. *S. enterica* subspecies are differentiated on the basis of biochemical traits and genomic relatedness. For example, we say *S. enterica* serovar Typhimurium and *S. enterica* serovar Enteriditis.

The *Salmonella* genus contains over 2,541 serovars!!!

Salmonella infections of man:

1. Enterocolitis (caused by many serotypes of *S. enterica*)

2. Enteric fever (known as typhoid and paratyphoid) caused by *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi

1.1.1 Enterocolitis (Gastroenteritis)

Pathogenesis - Severity of infection and disease influenced by:
- dose of ingested organisms (a minimum of 10^5 is usually required for symptomatic infection)
- state of host; highest incidence in young (<5 years), elderly, and in individuals with predisposing conditions such as malnutrition, impaired immunity, underlying infection
- virulence of infecting strain

Short incubation period (6-48 h; usually 8-12 h); multiplication in the mucosa of small intestine and colon, leading to inflammation with mononuclear response.

Clinical:
- nausea, vomiting, profuse diarrhoea, abdominal pain
- fever (38-39^{o}C), chills, headache, myalgia
- recovery within 2-3 days
- septicemia, rare complication in susceptible hosts

Laboratory diagnosis: stool culture

Epidemiology:

- infection by ingestion of food or drink contaminated by poor handling practices
- animal products (poultry, eggs, meat, milk) and animals are major sources
- person to person spread may occur (e.g., in nurseries)
- most cases occur in the home; institutional cases (e.g., nursing homes, schools) rank second
- many cases undiagnosed and unreported

Treatment

Antimicrobials are not recommended in uncomplicated *Salmonella* enterocolitis. Antibiotic treatment may prolong excretion of organisms in the stool and does not shorten the illness.

1.1.2 **Enteric Fever** (Typhoid and paratyphoid)

S. enterica serovar Typhi causes typhoid fever

S. enterica serovar Paratyphi causes paratyphoid fever

Enteric fever is a severe generalized infection whose principal feature is multiplication in the lymphoid tissue. Invasion of intestinal epithelium is followed by multiplication in mesenteric lymph nodes and entry into blood stream. Hyperplasia and necrosis of intestinal lymphoid tissue (Peyer's patches) may lead to ulceration, haemorrhage or perforation resulting in peritonitis, abscesses, etc. In untreated cases mortality is about 10%; survivors usually become (1) convalescent carriers excreting bacteria for up to 3 months or (2) chronic carriers (1-2% of cases) lasting over 6 months and occasionally lifelong.

Laboratory diagnosis is based on isolation of the organism from blood (1st week), stool and urine (2nd-3rd week).

Epidemiology - Human carriers are the only known reservoir. The infective dose* is about 10^6 and requires therefore previous multiplication in food or drink. Major vehicles are sewage-contaminated drinking water, shellfish, contaminated milk/milk products. Chronic carriers maintain endemic typhoid.

* A dose of 10^5 will infect about 25%; 10^9 will infect 95%.

Prevention is based upon sanitary disposal of human faeces, scrupulous cleanliness in handling food and purification and chlorination of water supplies. Available vaccines protect against small inocula and are therefore of low efficacy.

1.2 SHIGELLAE

Group of Gram-negative bacilli, generally non-lactose fermenters, found in the gastrointestinal tract of humans where they cause an acute diarrhoea (with mucus, pus and blood in severe cases). *Shigella sonnei* most common in Europe and North America (70-80%) and *S. flexneri* cause mild illness.

Shigella dysenteriae found mainly at the tropics causes severe illness. The invasion of the epithelial mucosa of the colon results in watery diarrhoea, cramps and fever. Infection follows ingestion of small numbers of the organism.

Epidemiology - Most frequent (2/3) in children below 10, under conditions of poor sanitation and crowding. Bacteria usually disappear from stools within 1 month; carrier state is generally short.

Prevention is similar to typhoid; no vaccines available.

1.3 ESCHERICHIA COLI

Gram-negative bacilli, lactose fermenters; most numerous aerobic species of the normal human intestinal flora. While harmless in the intestine, *E. coli* is potentially pathogenic elsewhere in the body (most frequent cause of urinary tract infection accounting for about 85% of uncomplicated bacteriuria).

E. coli isolates are serologically differentiated based on 3 major suface antigens: O (somatic), H (flagella) and K (capsule). To date, there are 173 O antigens, 56 H antigens and 103 K antigens.

Certain strains of *E. coli* are able to cause diarrhea. They are categorized into specific groups, known as pathotypes, based on virulence properties, mechanisms of pathogenicity, clinical syndromes, and distinct O:H serotypes. These groups include

- enteropathogenic *E. coli* (EPEC)
    - first pathotype to be described
    - can cause severe diarrhea in infants, especially in developing countries
- enterotoxigenic *E. coli* (ETEC)

- major cause of infantile diarrhea in developing countries
- most frequent agent responsible for “travelers’ diarrhea”

- enteroinvasive *E. coli* (EIEC)
  - non-bloody diarrhea and dysentery similar to *Shigella* species
- diffuse-adhering *E. coli* (DAEC)
  - associated with diarrhea primarily in young children (i.e., from 1-5 years)
- enteroaggregative *E. coli* (EAEEC)
- enterohemorrhagic *E. coli* (EHEC)
  - recognized as human pathogens in 1982 in hemorrhagic colitis outbreak
  - *E. coli* O157:H7 most common cause of EHEC-associated disease
  - verotoxins or Shiga toxins (since similar to Shiga toxin from *Shigella dysenteriae* type 1) associated to a sever and sometimes fatal condition, known as hemolytic uremic syndrom (HUS)

1.4 ENTEROBACTER SPP.

- may colonize and infect hospitalized patients
- causes wound infections, bacteremia and hospital-acquired pneumonia
- may be naturally resistant to antibiotics
- *E. sakazakii* linked to outbreaks of contaminated powdered infant formula
- *Enterobacter sakazakii* has been reclassified and are now referred to as *Cronobacter* species

1.5 OTHER ENTEROBACTERIACEAE

There is a large number of Gram-negative rods, free living or commensals of man or animals, which can cause infection in compromised hosts. Many of those infections are nosocomial. Among them are species of *Klebsiella*, *Enterobacter*, *Serratia*, *Acinetobacter*, *Citrobacter*, *Proteus,* etc.

2. VIBRIO AND CAMPYLOBACTER

The group includes *Vibrio cholerae* the cause of Asiatic cholera as well as *Campylobacter jejuni* and *C. coli*, common causes of enteritis in man.

2.1 *Vibrio cholerae*

A slightly curved (comma shaped) Gram-negative bacterium which produces an enterotoxin (cholera toxin) which binds to epithelial cells in the small intestine and causes them to secrete chlorides and to decrease absorption of sodium. Water accumulates in the lumen resulting in diarrhoea. The infection is endemic in Southeast Asia and parts of Africa, and is mainly waterborne.

2.2 *Campylobacter jejuni* and *Campylobacter coli* are normal flora in birds and domestic animals and a major cause of enteritis in man. Some strains appear to be invasive, others toxigenic. They cause fever, abdominal pain and bloody diarrhoea; possibly one of the agents of travellers' diarrhoea.

Stool cultures are inoculated on special media because the microorganism requires reduced oxygen tension and CO_2 (micro-aerophilic).

3. PSEUDOMONAS

Organisms of the genus *Pseudomonas* are free living gram negative rods, with the potential to cause illness in compromised patients. All*Pseudomonas* species tend to live in moist habitats and in water. The source of infection with these organisms is commonly humidifiers and other equipment containing water or other fluids capable of supporting the growth of the organism. There are many *Pseudomonas* species and the two most important are:

3.1 *Pseudomonas aeruginosa*

This is the most common *Pseudomonas* species. There are two clinical situations in which *P. aeruginosa* is particularly important as a pathogen. The first of these is as a respiratory pathogen in patients with cystic fibrosis and the second is in infections related to skin burns.

3.2 *Pseudomas cepacia*

This organism has the ability to multiply in very low nutrient environments and is therefore common organism contaminating saline and water. It is also found as a respiratory pathogen in cystic fibrosis patients.

All *Pseudomonas* infections are particularly difficult to treat because the organisms are intrinsically resistant to many commonly used antibiotics, and may acquire resistance to additional antibiotics.

4. HAEMOPHILUS INFLUENZAE

This organism is found as part of the normal nasopharyngeal flora of many individuals, including children. The organism is a significant pathogen in two situations.

i) It causes invasive infections, including meningitis, pneumonia, and joint infections, in children under five years. This type of infection is disappearing from the Canadian scene following the development of a vaccine which is now recommended as routine.

ii) *Haemophilus influenzae* is associated with exacerbations of infections in chronic bronchitis and is commonly isolated from sputum from these patients.

5. LEGIONELLA

This is an organism which grows in water and is commonly found in shower heads and water tanks. It is an opportunist pathogen, and in patients with deficiency in cell mediated immunity may cause a serious form of pneumonia. Exposure is by aerosol and person-to-person transmission does not occur.

6. HELICOBACTERACEAE

Helicobacter pylori

- Microaerophilic, spiral bacilli (non-spore-forming)
- most common cause of duodenal ulcers and chronic gastritis (inflamed tummy)
- interestingly, aspirin products rank second ☺
- bacteria isolated from ulcer craters
- feeding human volunteers causes ulcer formation and gastritis
- pepto-bismol, long used for gastritis, actually contains bismuth salts, known to inhibit *H. pylori*
- organism expresses urease, which raises the pH locally (protects from acidity of stomach)
- combination of antibiotics and proton pump inhibitor (triple therapy) appears most successful (amoxicillin, metronidazole and omeprazole)

7. ALCALIGENACEAE

Bordetella pertussis

- named in early 1900s by two scientists (Bordet and Gengou)
  "pertussis" means "violent cough"
- causative agent of whooping cough (by destroying the ciliated cells in trachea and bronchi)
- has 4 major virulence factors
  - pertussis toxin (A-B toxin)
  - extra cytoplasmic adenylate cyclase (weakens host cell's defense mechanisms)
  - filamentous hemagglutinin (attaches to ciliated cells of bronchi and releases exotoxin – does NOT actually invade the body *per se*)
  - tracheal cytotoxin – destroys ciliated epithelial cells and causes impaired clearance of bacteria, mucus buildup, and inflammatory exudates (probably major reason we

have violent cough!).

- current vaccine is heat-killed organism, including the pertussis toxin, filamentous hemagglutinin and adenylate cyclase (part of the DPT vaccine)

BIOCHEMICAL CLASSIFICATION

- ability to ferment lactose to gas and acid
- *E. coli* and most of Enterobacteriaceae ferment lactose, while *Salmonella*, *Shigella*, and *Pseudomonas* do **NOT**
- production of H_2S, ability to hydrolyze urea, liquefy gelatin and decarboxylate specific amino acids
- two important growth media are EMB and MacConkey agar
- EMB
    - Eosine-Methylene-Blue
    - Methylene blue inhibits Gram-positive
    - Lactose fermenters are deep purple to black
- MacConkey
    - Bile salts inhibit Gram-positive
    - Lactose fermenters develop pink-purple coloration

The enterics are also classified based on cell surface structures that bind specific antibodies. The enterics have 3 major surface antigens:

- O antigen – most external component of LPS
    - Differs from enteric to enteric
- K antigen – this is a capsule that covers the O antigen
- H antigen – is part of the subunits of the bacterial flagella (only motile bacteria have a H antigen)

TYPES OF DISEASES

1a. Diarrhea – with or without systemic invasion

a. No cell invasion – bacteria bind intestinal epithelial cells but do not enter the cell
b. Diarrhea caused by release of exotoxins (enterotoxins in the GI tract)
c. Enterotoxins cause electrolyte and fluid loss
d. Watery diarrhea without systemic symptoms (fever) – example includes *Vibrio cholera*

1b. Diarrhea with invasion of intestinal epithelial cells

e. Virulence factors allow binding and invasion of cell
f. Toxins released destroy the cells
g. Systemic immune response (fever)
h. Cell death results in bloody stools
i. Examples include *Escherichia coli* O157:H7

1c. Diarrhea with invasion of lymph nodes and bloodstream

j. Abdominal pain and diarrhea containing white and red cells
k. Fever, headache and increased white cell counts
l. Examples include *Salmonella enterica* serovar Typhi, *Yersinia enterocolitica*, *Campylobacter jejuni*

2. Various other infections

- Include urinary tract infections, pneumonia, bacteremia, sepsis

-The enterics are normal intestinal inhabitants. In hospital settings, nosocomial infections can arise. Examples include *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter*, *Serratia*, and *Pseudomonas aeruginosa*

MYCOBACTERIA

Group of bacteria including the causative agents of tuberculosis and leprosy characterised by a high content of lipids (waxes). The wax coat renders them more resistant to disinfectants and interferes with the Gram stain (i.e., most species, including the tubercle bacillus *Mycobacterium tuberculosis* do not take the Gram stain).

Mycobacteria are acid-fast; after staining with certain aniline dyes resist decolorisation by mineral acid. The ZIEHL-NEELSEN (Z/N) is a special procedure used for staining mycobacteria.

I. Mycobacterium tuberculosis

An acid fast bacillus causing a chronic, slowly progressing, most often pulmonary infection. The infection develops in stages:

1.1 Primary tuberculosis

After inhalation of aerosols containing tubercle bacilli (e.g., air in a room where a patient with advanced tuberculosis is coughing) - the bacteria reach the lung alveoli and start multiplying. Macrophages are mobilized, ingest them and move to the hilar lymph nodes; tubercle bacilli survive inside macrophages.

The complex formed by the local lesion in the lung, the draining lymphatics and the reacting (enlarged) lymph nodes is called the primary complex. This phase of infection is in the overwhelming majority of cases inapparent (i.e., the patient is unaware of the infection).

During the primary infection some tubercle bacilli escape in the lymphatics and blood stream and reach the lungs, bones, kidneys, meninges etc. where they produce microscopic foci of infection.

About 6 weeks after the onset the cell mediated immune (CMI) system becomes fully active and in the majority of cases stops the infection and walls in the microscopic lesions. The primary complex

heals as well as the foci resulting from dissemination. Tubercle bacilli, though, can survive in those foci (controlled by the CMI) and eventually reactivate months or years later. The latent infection and the active CMI is reflected in a positive tuberculin test.

Tuberculin is a protein obtained by concentrating and purifying the liquid culture medium in which Mycobacterium tuberculosis has grown. The crude preparation is called "old tuberculin" (OT) while the purified preparation now in use is the "purified protein derivative" (PPD).

In a small number of cases early dissemination results in pleural effusions, miliary tuberculosis and/or meningitis; occasionally primary infection may progress to tuberculous pneumonia.

1.2 Post-primary tuberculosis

Post-primary tuberculosis is usually a late reactivation of silent lesions (lungs, kidneys, bones, etc.) in hosts who have developed a certain degree of immunity (tuberculin positive). It occurs in a small percent of those infected (about 5% in developed countries). An increased proportion is found in AIDS. Reinfection (infection with a different strain) occurs rarely. Post-primary tuberculosis runs a chronic course.

1.3 Immunity in tuberculosis

Cell mediated immunity (CMI) is the most important reaction to *M. tuberculosis*, evidenced by a positive tuberculin test. The most widely used tuberculin test is the **Mantoux test** in which 0.1 ml of a tuberculin solution (PPD standardised to contain 5 TU tuberculin units) are injected ***strictly intradermally*** into the medial area of the forearm.

Reading the Mantoux test - widest transverse diameter of induration (disregarding redness) is recorded in millimeters (mm) after 48 - 72 hours. Results are interpreted according to the size of induration:

10 mm or larger - positive tuberculin test

5 - 9 mm - doubtful; in most cases cross reactions with other mycobacteria.

<4 mm - negative

N.B. A positive tuberculin test indicates that the individual has been infected with *M. tuberculosis* at some time; it does not necessarily diagnose an active infection.

1.4 Laboratory diagnosis includes:

1.4.1 Microscopic examination of sputum smears, an important step in the presumptive diagnosis because of the long time taken by the cultures. Smears are stained by fluorescent dies or by Ziehl-Neelsen.

1.4.2 Cultures on special media (e.g., Lowenstein-Jensen) take usually 3-6 weeks to show growth and are generally kept for up to 8 weeks. Positive cultures are checked for sensitivity to antituberculous drugs.

2. NON-TUBERCULOUS ("ATYPICAL") MYCOBACTERIA

Non-tuberculous mycobacteria are occasionally isolated from patients with chronic pulmonary disease indistinguishable from tuberculosis (e.g., *M. kansassi, M. avium, M. intracellulare*). These mycobacteria (also referred to as "atypical mycobacteria") show higher resistance to antituberculous drugs. *M. scrofulaceum* is a common cause of lymphadenitis (especially cervical) in children.
Skin infections are produced by *M. marinum* and soft tissue abscesses by *M. fortuitum*. Non-tuberculous mycobacteria usually give rise to a doubtful positive (5-9 mm) tuberculin test. Infections with non-tuberculous mycobacteria are seen with increased frequency in immunocompromised/immunosuppressed patients (e.g., in AIDS), particularly generalized infection with *M. avium* or *M. intracellulare*.

3. Mycobacterium leprae

-The causative agent of leprosy (Hansen's disease)

-Two types of leprosy: tuberculoid leprosy and lepromatous leprosy

3.1 Tuberculoid leprosy

-few erythematous plaques, having flat centres and raised edges; peripheral nerve damage with complete sensory loss; nerve enlargement is visible.

-infected tissues have many lymphocytes and granulomas, but very few bacilli

-low infectivity

3.2 Lepromatous leprosy

-many erythematous macules, papules and nodules; severe tissue destruction; patchy sensory loss; no nerve enlargement.

-infected tissues have many bacilli present

-high infectivity

3.3 Epidemiology

-not common in North America, mostly seen in Asia and Africa

-between 1-2 million cases currently reported worldwide; only 125 new cases seen in US each year.

-transmission is primarily human to human through respiratory droplets; in some rare cases transmission occurs through an animal reservoir (armadillos).

3.4 Diagnosis

-microscopy is used for the lepromatous diagnosis, but not for the tuberculoid form since so few bacilli are present; skin testing is used for the tuberculoid form.

-culture is not used for diagnosis

3.5 Treatment

-Tuberculoid form: Dapsone combined with rifampin

-Lepromatous form: Dapsone, rifampin and clofazimine

-Prompt recognition and treatment is necessary to prevent and control the spread of the disease.

SPIROCHETES

Spiral-shaped, gram-negative, microorganisms including the genera:

Treponema causing syphilis, yaws, pinta

Leptospira causing leptospirosis

Borrrelia causing relapsing fever, known as Lyme disease

1. *Treponema pallidum*

Spiral-shaped, non-cultivable in vitro, practically invisible when stained with the routine procedures used in microbiology (Gram, Giemsa, Ziehl-Neelsen). Visible on darkfield microscopy.

1.1 Syphilis

Syphilis is the infection caused by *T. pallidum* is most often a sexually transmitted infection with a chronic evolution in several stages:

Primary syphilis characterized by the appearance of a sore (chancre) 3-4 weeks after the infecting contact. Darkfield examination of the fluid oozing from the lesion shows presence of treponemes.

Secondary syphilis about 6 weeks after appearance of chancre, with generalized or localized rash, mucous lesions (oral, perineal, anal) abounding with treponemes and generalized lymphadenopathy.

After primary or secondary phase, spontaneous remission may occur

Latent syphilis follows the secondary stage. The infected individuals have no manifestations of infection and after 4 years the infection is rarely communicable. Pregnant women, though, can transmit it to the foetus (congenital infection).

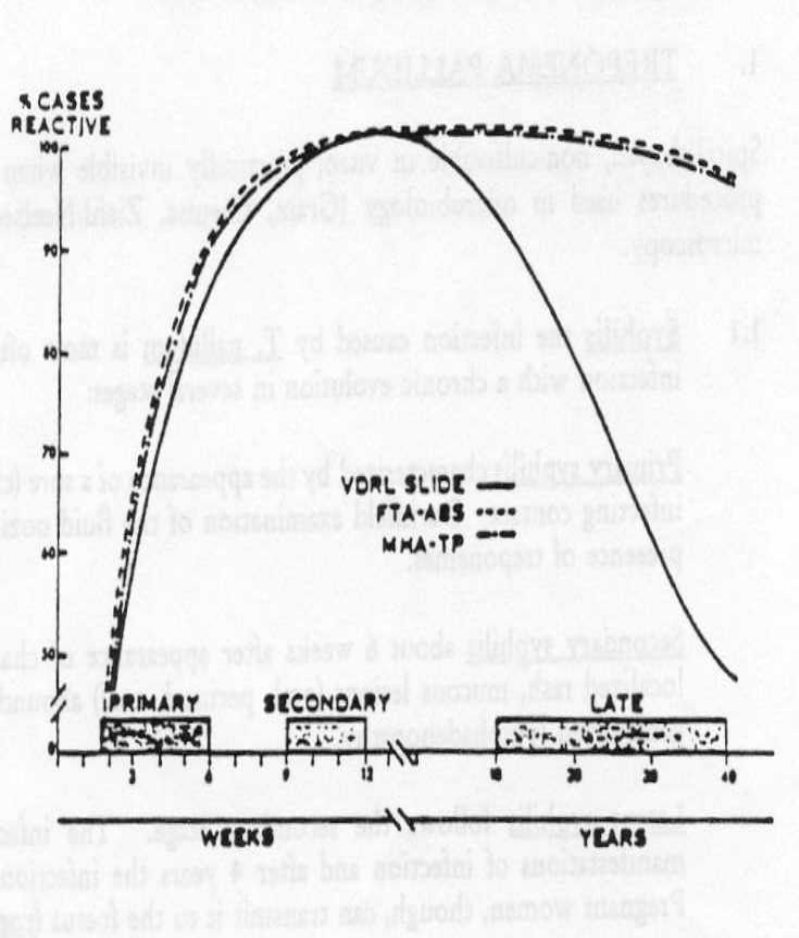
Late syphilis - generally due to obliterative endarteritis can involve skin and mucosae, cardiovascular or nervous system, etc. All tissues may be involved.

Serology is the main diagnostic tool, becoming positive during the primary stage, reaching practically 100% during the secondary and latent stage. Slight decline in late syphilis but vast majority still positive. There are two types of tests used for syphilis serology:

1. Non-treponemal tests- using a cardiolipin antigen not extracted from treponemes (i.e., non-specific). The tests (VDRL, RPR, Wassermann):
   - are used as screening tests
   - are positive early
   - after successful treatment become negative or fall in titer

2. Treponemal tests - use whole treponemes or treponemal extracts (i.e., specific). In Ontario two tests are commonly used:
   a) FTA-ABS - fluorescent treponemal antibody absorption
   b) MHA-TP - microhaemagglutination *T. pallidum*; routinely used for confirmation of positive VDR.

Figure: Serology of untreated syphilis



Microscopy - darkfield microscopy can be used to identify the organisms from the exudate of the lesions of the patient: BUT it must be done immediately in order for the organism to remain active

Culture - culture methods are not available; organism does not grow in cell culture assays

Prevention - Education about the risk of sexually transmitted infection and preventive use of condoms

- Periodic serological testing of individuals at high risk with subsequent treatment (including testing during pregnancy)

2. LYME DISEASE

Lyme disease is an infection caused by the spirochete, *Borrelia burgdoferi*, which is transmitted by a tick bite. It is usually transmitted by Ixodes ticks which usually have a 2 year life cycle with 3 stages (larva, nymph, and adult). The disease is present in many parts of the U.S. but is rare in Canada. Clinically there may be involvement of the skin, joints, nervous system, or in the heart. Diagnosis is usually confirmed by serologic testing but a positive result is not always diagnostic of infection but must always be interpreted in light of the clinical picture.

2.1 Clinical Diagnosis

- patient has either (or both) of the following:
- erythma migrans
- one type of "late" manifestaton (musculoskeletal, nervous system of cardiovscular).

2.2 Laboratory Diagnosis

- Microscopy: not recommended; *B. burgdoferi* is rarely seen in clinical specimens
- Culture: *B. burgdoferi* is difficult to culture and requires specialized media; this method of identification is rarely used.
- Serology: Most commonly used for diagnosis
    - -immunofluorescence assay and ELISA are most commonly used; ELISA is preferred because it is better at identifying all stages of the disease; EXCEPT in the early stages...all serological tests are fairly insensitive for the first 2-4 weeks of infection.

2.3 Treatment and Prevention

- Early Lyme disease is treated effectively with doxycycline, amoxicillin or cefuroxime.
- Those with neurologic and musculoskeletal manifestations must undergo prolonged treatment.
- To prevent Lyme disease, avoid ticks and their natural habitats and wear protective clothing.
- Vaccine for *B. burgdoferi* is available; directed against the ospA antigen of the organism.

3. *Leptospira interrogans*

-can cause sub-clinical infection, mild flu-like illness or a severe systemic disease (Weil's disease- renal, hepatic failure, extensive vasculitis, myocarditis and death). Severity is dependant on number of infection organisms, the hosts immune system and the virulence of the strain

-thin and highly motile; enter the body through small cuts and abrasions; disseminate into the bloodstream and infect all tissues, including the CNS.

-few cases in North America

-humans are accidental end-stage hosts; infection from exposure to water contaminated with urine from an infected animal (animal reservoirs: rodents, dogs, farm animals etc.)

-Treatment: IV penicillin or ampicillin for sever infections; oral ampicillin, amoxicillin or doxycyline for milder infections.

CHLAMYDIAE

These are very small bacteria which are obligate intracellular energy parasites (i.e., cannot be grown on artificial media and cannot produce their own ATP). They have a unique life cycle with 2 forms: infectious elementary body (300-400 nm) and reticulate body (800-1000 nm).

There are 3 main species:

1.1 *Chlamydia trachomatis*

C. trachomatis is the most common sexually transmitted disease in Canada and the U.S. It is a major cause of urethritis in males ("non-gonococcal urethritis") and cervicitis in females. Many infected patients are asymptomatic and undetected due to limitations of present diagnostic methods. Untreated, the infection may spread producing prostatitis and epidiymitis in males and pelvic inflammatory disease (PID) in females. Women bear the major burden of disease as a result of complications of PID which include tubal infertility, ectopic pregnancy, and chronic pelvic pain.

-species has been divided into 2 biovars: trachoma and LGV (lymphogranuloma venereum)

-trachoma consists of 15 serovars and LGV has 4 serovars

Other infections caused by *C. trachomatis*:

- Trachoma - a chronic ocular infection; leading cause of blindness in the middle-East, North Africa, and S.E. Asia.
- Inclusion conjunctivitis and less frequently pneumonia in the newborn as a result of perinatal transmission
- Lymphogranuloma venereum - a sexually transmitted disease caused by particular serotypes of *C. trachomatis* resulting in inguinal lymphadenopathy. Endemic in tropical and subtropical countries.

1.2. ***Chlamydia pneumoniae*** causes respiratory tract infections ranging from pharyngitis to

pneumonia (most of them mild; major cause of atypical pneumonia). A large proportion of infections is subclinical. Probably spread by aerosols (droplets of respiratory secretions).

1.3 ***Chlamydia psittaci*** is primarily a bird pathogen but may be transmitted to humans resulting in pneumonia or systemic infections such as endocarditis.

MYCOPLASMAS

These are the smallest free-living bacteria (i.e., can grow outside cells and on artificial media), 100 - 300 nm in size. Many species of mycoplasma are found as saprophytes, being part of the normal flora of the oropharynx and genital tract of humans and animals. Some species are pathogenic in humans.

1. *Mycoplasma pneumoniae*

M. pneumoniae produces primarily acute respiratory disease and is the most common cause of atypical pneumonia. Infections are most common in the younger age groups (15 - 35 years). Rarely it may cause more serious infections such as meningoencephalitis or myocarditis. Although the diagnosis may be confirmed by serology, most cases of respiratory illnesses presumed to be due to *M. pneumoniae* are diagnosed on clinical grounds with no laboratory confirmation.

It is estimated that this organism causes 2 million cases of pneumonia and 20 million cases of other respiratory infections in the United States.

Treatment includes erythromycin or tetracycline

2. Genital mycoplasmas

Mycoplasma hominis and *Ureaplasma urealyticum* are part of the normal genital flora. Colonization rates increase in adults in relation to the number of sexual partners. They may cause urethritis, epididymitis, pelvic inflammatory disease, and postpartum fever. Their role in infertility and prematurity remains controversial.

MYCOTIC (FUNGAL) INFECTIONS

In the modern world of AIDS, organ transplantation, and chemotherapy, immunocompromised people will be on the rise. This will result in a lowered cell-mediated immunity, and will allow you to see an increase in the incidences of fungal infections of every type. Fungi are eukaryotic organisms, lack chlorophyll and so cannot generate energy through photosynthesis. They need an aerobic environment. The following definitions are useful:

Dimorphic fungi:	fungi that grow as either yeast or mold (depends on temperature, environmental conditions, etc.)
Hyphae:	threadlike, branching tubules composed of fungal cells attached end to end
Molds (mycelia):	multicellular colonies composed of clumps of intertwined and branching hyphae
Saprophytes:	fungi that live and use organic matter such as soil, rotten vegetation as the energy source
Spores:	reproducing bodies of molds
Yeast:	unicellular growth form of fungi. They can appear spherical to ellipsoidal. They reproduce by budding (if they do not separate, they form long chains of yeast cells known as pseudohyphae).

It can be helpful to remember the fungal diseases by the depth of the skin that they infect:

[skin]- Superficial – Cutaneous – Subcutaneous – Systemic - [bloodstream]

1. Superficial Fungal Infections

- pityriasis versicolor (multicolored) caused by *Malassezia furfur* – hypo or hyperpigmented patches that remain white if you suntan
- tinea nigra (black colored) caused by *Exophiala werneckii* – painless patches on soles of hands and feet
- pigment change of the skin
- treatment for both consists of spreading dandruff shampoo containing selenium sulfide over skin

2. Cutaneous fungal infections (skin, hair, nails)

- dermatophytoses are a category of cutaneous fungal infections caused by more than 30 species of fungi
- dermatophytic fungi live in dead, horny layer of skin, hair and nails
- secrete enzyme keratinase (digests keratin), thereby resulting in scaling of skin, loss of hair and crumbling of nails.
- Common ones include *Microsporum*, *Trichophyton* and *Epidermophyton*
    - *Tinea corporis* (body) – ringworm (looks like ring-shaped worm under skin)
    - *Tinea cruris* (jock itch) – itchy red patches on groin and scrotum
    - *Tinea pedis* (athlete's foot) – commonly begins between toes
    - *Tinea capitis* (scalp) – primarily in children, seen as scaly red lesions with loss of hair
    - *Tinea unguium* (onychomycosis) – nails are thickened, discolored and brittle
- treatment usually topical imidazoles (keep skin dry)
    - *Candida albicans* – can infect the mouth (oral thrush) groin (diaper rash) and vagina (vaginitis)

3. Subcutaneous fungal infections

- *Sporothrix schenckii* – causes sporotrichosis, an occupational hazard for gardeners
    - Subcutaneous nodule appears and becomes necrotic and ulcerative
    - Ulcer may heal but new nodules will arise nearby and along the lymphatic tracts of the arm
    - Treatment with potassium iodide or amphotericin B
- *Phialophora* and *Cladosporium*
    - Cause chromoblastomycosis
    - Found on rotting wood, infection follows puncture wound
    - Violet-colored wartlike lesions (cluster of lesions resemble cauliflower)
    - Treatment with itraconazole and local excision

4. Systemic fungal infections

- Dimorphic fungi that grow as mycelial forms, with spores at 25°C on Sabouraud's agar
- At 37ºC, on blood agar plates, they grow in a yeast form
- This dimorphism is part of the human infection (in soil, they are mycelia and release spores in air; once inhaled by humans they grow as yeast cells)
- Three fungi that cause systemic disease in humans are *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides immitis*

- All three have similar mechanism of disease

    - Acquired by inhalation (inhaled as spores) but are NOT transmitted person to person (like TB)

    - Local infection in lung, then bloodstream dissemination

    - 3 clinical presentations
        - Asymptomatic
        - Pneumonia
        - Disseminated

- *Cryptococcus neoformans*
    - Polysaccharide encapsulated yeast (not dimorphic though)
    - Inhaled in lungs, usually asymptomatic
    - Major manifestation is meningoencaphalitis
    - Most cases occur in immunocompromised persons (about 10% of AIDS patients develop cryptococcosis)
    - Treatment with amphotericin B and flucytosine
    - Treatment may last for 6 months (AIDS patients may need for life)

Aspergillus flavus

- spores of this mold are everywhere
- some develop type 1 hypersensitivity reaction (IgE-mediated)
- persons with lung cavitations from TB or malignancies can get aspergillus fungal ball (aspergilloma) in cavity
- immunocompromised persons can develop invasive pneumonias and disseminated diseases
- *A. flavus* and other fungi produce toxins called mycotoxins
- *A. flavus* produces aflatoxin
    - Contamination of peanuts, grains and rice

PARASITES AND PARASITIC INFECTIONS

A. PROTOZOA (UNICELLULAR)

1. Giardia lamblia

Disease: Giardiasis; infection of the small bowel, often asymptomatic but also associated with various intestinal symptoms (especially diarrhoea and steatorrhea). Incubation: 1-4 weeks.

Diagnosis: Identification of cysts in faeces by microscopy.

Epidemiology: Trophozoites in the bowel become cysts eliminated with stools and then accidentally ingested. Carriers important in transmission. More frequent in areas of poor sanitation and in children (especially tropical countries).

Prevention: Health education and sanitary disposal of faeces. Effective water treatment.

2. Trichomonas vaginalis

Disease: Vaginitis (Trichomoniasis) with itching, foul smelling, sometimes frothy discharge. In males usually asymptomatic; occasionally urethritis, prostatitis.

Diagnosis: Wet-mounts → microscopic identification of trichomonads in vaginal or urethral discharge. Can also be cultivated.

Epidemiology: World-wide distribution. Transmitted almost exclusively through sexual contact.

N.B. In order to avoid reinfection, investigation and treatment of partners is necessary.

3. **Entamoeba histolytica**

Disease: Amoebiasis (amoebic dysentery); infection of the large intestine which may be asymptomatic or manifest (diarrhoea/constipation; acute or chronic dysentery). May spread via the blood to produce liver, lung or brain abscesses.

Diagnosis: Identification of trophozoites or cysts in faeces or lesions by microscopy.

Epidemiology: Worldwide in areas of poor sanitation (especially tropical countries). Clinical disease is prevalent in warm countries. Spread by water, contaminated vegetables (especially raw), flies, food handlers.

Prevention: Education, sanitation. Effective water treatment. Wash fruits and vegetables.

4. **Toxoplasma gondii**

Disease: Toxoplasmosis; infections usually mild, often undetected. May present with fever, lymphadenopathy, rarely severe. Congenital infection may be very serious: hydrocephaly, microcephaly, psychomotor disturbances, chorioretinitis, etc.

Diagnosis: (1) serological; (2) mouse inoculation.

Epidemiology: Acquired through ingestion of raw or undercooked meat (e.g., pork) or by contact with cat faeces. Other animals can also be infected (rodents, cattle, sheep, chickens).

Prevention: Sanitation, thorough cooking of meat. Wash fruits and vegetables, wash hands after gardening.

5. Plasmodium spp.

A group of four protozoa causing different types of malaria: Plasmodium vivax, P. malariae, P. falciparum, P. ovale.

Disease: Malaria in the typical case is characterized by recurrence of chills, sweating, headache. May lead to jaundice, renal failure, acute encephalitis and coma. Secondary anaemia; enlarged spleen. The interval of recurrences varies with the organisms causing the infection.

Diagnosis: Blood smears (thin and thick) stained with Giemsa.
N.B. Collect blood just before fever peaks or if peak unknown, at time of seeing the patient and at 6 hour intervals thereafter.

Epidemiology: Sporozoites of plasmodia introduced through bite of anopheline mosquitos. Occasionally through transfusion from infected donors; drug addicts.

Prevention: Eradication or control of mosquitos; treatment.

Prophylaxis: Preventive treatment with antimalarials should start before reaching endemic area and continue after returning for recommended period.

6. *Cryptosporidium* spp. (*C. parvum, C. hominus* and other species can infect humans)

Disease: Cryptosporidiosis may result in diarrhea, vomiting, anorexia, malaise. May be life threatening in immunocompromised individuals.

Diagnosis: Identification of oocysts in faecal specimens.

Epidemiology: Transmission of oocysts may occur through the faecal-oral route, water or food. Massive waterborne outbreaks reported. Cattle are an important animal reservoir.
Prevention: Public health education (hand washing) and sanitary disposal of faeces.

Restrict access of cattle to source water

7. ***Cyclospora cayetanensis***

Disease: Cyclosporiasis may result in prolonged and severe diarrhea.

Diagnosis: Identification of oocysts in faecal specimens.

Epidemiology: Transmission of oocysts may occur through contaminated water or food. Recent foodborne outbreaks reported in North America. Incubation time one week.

Prevention: Public health education (hand washing) and sanitary disposal of faeces. Effective water treatment.

B. METAZOA (MULTICELLULAR)

1. NEMATODES (Round worms)

1.1 *Enterobius vermicularis* (**Pinworm**)

Disease: Enterobiasis (Pinworm disease, oxyuriasis) - mild infection of caecum/colon. May cause itching (pruritus ani) leading to disturbed sleep, irritability, scratching, may cause secondary infections.

Diagnosis: "Scotch-tape" swabs of anal area used for microscopic identification of typical ova (eggs). Occasionally adult worms (2-13 mm) can be seen.

Epidemiology: Highest incidence in school-age children. Infective eggs transmitted directly (hand to mouth) or indirectly through clothing, bedding, food etc. contaminated with eggs.

Prevention: Education (handwashing after use of toilet or before meals, discourage nail-biting and direct scratching of anal area). Frequent bathing (preferably showers) with regular change of underclothing, night clothes and bed sheets. Reduction of over-crowding in living accommodation.

1.2 *Trichinella spiralis*

Disease: Trichinosis - caused by the migration through the body of larvae of *Trichinella spiralis* and their subsequent encystment in muscles. Characteristic orbital oedema, muscle pains, headache, fever, weakness.

Epidemiology: Ingestion of raw or insufficiently cooked meat containing viable, encysted trichinae (pork, bear).

Prevention: Proper cooking of pork (or bear meat). Storing at -27 ^{o}C for 36 hours kills trichinae.

1.3 ***Ascaris lumbricoides***

Symptoms: Vague abdominal discomfort (pain, vomiting, obstruction).

Diagnosis: Stool examination for eggs.

Treatment: Mebendazole or pyrantel pamoate surgery to clear worm bolus.

1.4 ***Anisakis simplex*** **(Herringworm)**

Definite hosts: Dolphins, porpoises, whales.

Intermediate hosts: Salmon, mackerel, cod, herring, tuna, squid.

Symptoms: Abdominal pain, nausea, vomiting.

Diagnosis: Spontaneous expulsion, laparotomy, endoscopic or radiologic examinations, immunological tests.

Treatment: Spontaneous expulsion, surgical resection, endoscopy.

2. PLATYHELMINTHS (Flat worms)

2.1 CESTODES (Tapeworms)

Diphyllobothrium latum (Fish tapeworm):	3-10 m long
Taenia saginata (Beef tapeworm):	about 4-5 m, up to 25 m long
Taenia solium (Pork tapeworm):	2-7 m long

Disease: Infection with adult tapeworm→ nervousness, loss of appetite, insomnia, weight loss, abdominal pain; sometimes asymptomatic. Infection with larvae (cysticercosis) → severe

disease involving organs/tissues in which encystment occurs.

D. latum - infection can cause anemia (i.e., competitor for vitamin B12)

Diagnosis: - Examination of stools for presence of proglottids. Microscopic examination of faeces for presence of eggs.

Epidemiology: Ingestion of raw/insufficiently cooked meat or fish containing larvae → tapeworm. With *T. solium*, ingestion of eggs can in some rare cases lead to cysticercosis (i.e., brain abscesses).

Prevention: Education. Stop access of swine to human faeces. Cook meat/fish well. Sanitation.

2.2 TREMATODES (Flukes)

***Schistosoma* spp.** (Blood fluke)

Disease: schistosomiasis may result in rash/itchyness, fever, muscle aches, lymphadenopathy, enlargement of liver and spleen.

Diagnosis: Microscopic examination for eggs in faeces or urine

Epidemiology: swimming in (or contact with) fresh water containing infected snails. Free-swimming cercaria penetrate the skin.

Prevention: Avoid contact with ponds, lakes etc. in endemic regions. Control of snail populations. Sanitation.

VIRUSES AND VIRUS INFECTIONS

GENERAL CHARACTERISTICS OF VIRUSES

- Grow only in living cells

- Possess only one type of nucleic acid (either DNA or RNA)

- Multiply by separate synthesis of nucleic acid and protein which are then assembled to form virus particles. Viruses do not divide.

- Do not have own metabolism = need to grow within cells

- Vary in size from l0 nm-300 nm (l nm = l nanometer = l/l,000,000 mm = approximately l/2,500,00 inch)

Basic components:

l. nucleic acid core - carries the genetic code

2. protein coat - protects the core, carries the antigens

Some contain in addition carbohydrates or lipids.

Viruses can be grown in:

l. cell cultures

2. embryonated eggs

3. living animals

N.B. Not all viruses grow in all three.

Replication (multiplication):

1. Adsorption - Viruses adsorb to receptors on the cell surface.
2. Penetration and uncoating - The virus enters the cell, the protein coat is lost and the nucleic acid released; it becomes undetectable (eclipse).
3. Synthesis of nucleic acid and protein - The viral nucleic acid redirects the cell metabolism to produce viral protein and nucleic acid.
4. Assembly-Maturation - Viral components are assembled to form mature (infective) virus particles.
5. Release - Newly formed virus particles are released by either lysis (destruction) of the cell or budding through the cell membrane.

Detection of viruses in the virus diagnostic laboratory: the golden rule is that viruses die out quickly in clinical specimens. They must be examined and inoculated in the laboratory within hours after collection. Use viral transport media and courier services, no room for postal services. Do not freeze, but keep refrigerated.

There are 2 main approaches to viral diagnosis:

A. Detection of viruses in clinical specimens.

B. Detection of the immune reactions triggered by the virus in the patient, i.e., detection of antibodies in the patient's blood.

A. Detection of viruses in clinical specimens.

1. Visualization by electron microscopy (E.M.), since viruses are invisible by light microscopy and very high magnification required. Only used for a few viruses which happen to be present in very large numbers in clinical specimens.

2. Effects after inoculation into cell cultures: this requires multiplication, i.e., time.

- cytopathic effect: visible modifications of infected cells
- hemagglutination: some viruses can agglutinate red blood cells added to the cell cultures
- immunofluorescence and other serological methods will reveal the presence of viral antigenic material within the infected cell culture, even if there is no visible damage caused by the multiplying virus

3. Direct detection of viral antigens in clinical specimens themselves by immunological methods.

B. Detection of the immune reactions triggered in the patient's immune system, i.e., detection of antibodies in the patients's blood.

1. presence or absence of antibody against a given virus = **immunity tests**

2. rise in antibody or high antibody titer against a given virus = evidence that this particular virus has recently caused a viral illness = **diagnostic tests**.

VIRUSES CAUSING DISEASE IN HUMANS

1. RESPIRATORY VIRUSES

A group of viruses which are found mainly in the respiratory tract and produce respiratory tract disease. They belong to different virus families.

Included in this group are:

1. influenza viruses
2. parainfluenza viruses
3. respiratory syncytial viruses
4. rhinoviruses
5. adenoviruses

l) Influenza viruses:

- Two main types: A - causes major epidemics
  B - causes milder disease; occasionally occurs in epidemics

- Grow easily in certain cell cultures.

- Produce a haemagglutinin (i.e., agglutinate red blood cells).

The nucleic acid is made of separate but connected segments and is subjected to frequent recombination. The resulting antigenic variability explains the periodic appearance of new types which spread throughout the world causing "pandemics" of influenza.

Clinical: - Acute febrile illness with variable respiratory symptoms.
- The extreme age groups (infants and elderly) are particularly sensitive to both disease and complications.

Diagnosis: Throat washings or naso-pharyngeal aspirate taken within 3 days after onset and inoculated immediately in cell cultures. If delay, use transport medium.

If patient is unable to gargle - swabs may be taken instead.

Serum:

- two blood samples ("paired sera") taken at 2-3 weeks interval ("acute" and "convalescent" phase), are investigated for increase in titer of antibodies. Acute sample to be collected as soon as possible after onset.
- Serum (sera is plural) is the liquid portion (containing antibodies) of whole blood when cells are removed. If the only test possible is detection of specific IgG or total antibody, then paired sera are required. Usually, sera is taken 14-21 days apart are tested at the same time. A rise in amount of antibody (known as titre) indicates a current infection. simultaneously. If there is a significant rise/decrease/seroconversion in titer (amount of antibody), your patient is considered to have a current infection. NOTE: testing for a single IgM? Then a single serum sample is sufficient.

Prevention: - Vaccines, usually combined against both types, are available but their use is hampered by the continuous antigenic changes. Antibodies against one strain protect only partially or not at all against newly appeared strains. Vaccine is administered to high risk groups (elderly, heart diseases, health workers).
- some antiviral drugs (amantidine) are available but are only active against type A.

2) **Parainfluenza viruses**. Frequent in infants and small children. Cause respiratory disease that may result in serious complications. Cause laryngotracheobronchitis (croup), bronchiolitis and bronchopneumonia. No vaccine available.

3) **Respiratory syncytial virus**. Major respiratory pathogen in children under 2 years: bronchiolitis and pneumonia which may occasionally be fatal. Can cause epidemics. No vaccine available. Antiviral: ribavizine.

4) **Rhinoviruses**. Main cause of common colds. There are over l00 different serological types and no cross immunity; repeated infections are therefore common.

5) **Adenoviruses**. Cause mainly pharyngitis and/or conjunctivitis; more frequently in children;

inapparent infection is common. Some types may cause pneumonia in young infants and children. Can cause epidemics. Vaccines used in the army.

6) **Other viruses causing occasional upper respiratory tract infections**:

- echoviruses
- coxsackieviruses
- herpesviruses

Laboratory diagnosis of respiratory viruses.

A. Detection of virus:

1. Fastest approach is the direct detection of viral antigens by immunofluorescence or other immunological methods, combined with inoculation in cell cultures. Positive results in as little as one day.

2. Clinical specimens, by order of decreasing usefulness: naso-pharyngeal aspirates, throat washings, throat and nasal swabs.

3. Chances of detecting the virus decrease rapidly after a few days illness.

B. Serological diagnosis:

Less useful, and usually retrospective. Dates of paired sera in relation to illness important for the lab. The laboratory will test for a series of respiratory agents, since symptoms are rarely typical for a given virus.

Practical importance of rapid laboratory diagnosis of respiratory viruses.

A. Public health measures

1. detection of circulating strains and antigenic make-up of influenza strains in the community

2. decide if amantadine is useful (active against influenza A only)

3. restricting admissions/closing of nursing homes, hospitals, etc.

B. Management of patients

1. Allows cohorting in pediatric facilities for RSV and croup.

2. Restrictive use of antivirals (amantadine for influenza A, ribavirine for RSV).

3. Special care for debilitated patients.

2. ENTERIC VIRUSES

This group includes Enteroviruses (polioviruses, coxsackieviruses, echoviruses). All multiply mainly in the gastro-intestinal tract, but, as a rule, do not cause gastro-enteritis.

However, from the GI tract, they can reach other target organs, as a result of viremia (presence of virus in the blood) and can cause severe damage, usually in the central nervous system (see viruses infecting the central nervous system).

Common Characteristics:

Infection is acquired through the respiratory tract (naso-pharynx) and/or gastro-intestinal tract.

The infection with enteroviruses is:

- in approx. 95% of cases, inapparent, i.e., the virus multiplies for up to 5-6 weeks in the intestine without causing any symptoms.

- in approx. 4-5% - minor illness (fever, pharyngitis, etc).

- in 1% or less - major illness (e.g., paralysis, aseptic meningitis, myocarditis, etc).

Main Groups of Enteroviruses:

1. **Polioviruses**:

- Three immunological types: type 1, 2 and 3.

- Man is the only natural host.

Diagnosis: 1. Isolation of the virus from stool specimens (virus in stools up to 5-6 weeks), CSF,

pericardial fluid, etc. after inoculation in cell cultures.

2. Serology: Two specimens of serum (acute phase and convalescent phase) are necessary. The acute specimen should be obtained immediately after the onset of the disease. The convalescent specimen, two to three weeks later.

Epidemiology: The most important factor which favours the spread of the disease is the large number of inapparent infections (people infected with enteroviruses, show no sign of disease but spread the virus to other susceptible persons).

Prevention: Two types of highly effective vaccines are used:

1. Killed (inactivated) polio vaccine (Salk) (trivalent)

Does not produce local immunity (IgA) in the gastro-intestinal tract; therefore, although the individual is protected, the virus can multiply in his G.I. tract and spread in the community. This vaccine has now been superseded by the live attenuated vaccine. The killed vaccine is still used for immunocompromised individuals, and adults who have never been immunized against poliomyelitis.

2. Live attenuated polio vaccine (Sabin) (trivalent):

- easy to administer (by mouth).
- produces IgA as well as IgG, therefore produces intestinal tract resistance to infection and prevents the spread of wild virus in the community. Should not be administered to immunocompromised patients or adults who have never been vaccinated against polio before.

2. Coxsackieviruses:

Divided in two groups: A - several types
B - several types

Clinical: - minor respiratory illness (mainly group B)
- aseptic meningitis (both group A and B)
- herpangina and hand-foot-and-mouth disease (group A)
- pleurodynia, pericarditis and myocarditis (group B)

Epidemiology: - seasonal variation (summer and fall)
- the prevalent type varies every few years

Diagnosis: - same as for polioviruses
- most group A coxsackieviruses can be isolated only by mouse inoculation

Vaccine: - none

3. **Echoviruses:** (ECHO = enteric cytopathogenic human orphan viruses)

- so named, because, when first isolated in cell cultures, no relation to disease was evident
- several types

Clinical: - minor respiratory illness
- aseptic meningitis

Diagnosis: - same as for poliomyelitis

Vaccine: - none

3. VIRUSES CAUSING DIARRHEA

A. Rotavirus

Virus causing epidemics of diarrhea of infants, during winter months. The virus multiplies in the small intestine, producing a loss of fluids and electrolytes, as well as a transient malabsorption of fats and sugars.

Clinical:

Acute gastroenteritis in infants and young children (6 months - 2 years) with diarrhea, vomiting, fever, dehydration. Rotaviruses are found in enormous numbers in stools, diapers, on hands, surfaces, etc. and are highly infectious.

Diagnosis: Identification of the virus present in stools by electron microscopy or immunological techniques. Cell cultures are of no practical value. Examine stools within 3 days.

Epidemiology: Short incubation period: 2-3 days.

Transmission from infants with explosive diarrhea by the fecal-oral route, via aerosols and fomites. Nosocomial outbreaks occur in nurseries, day care centers and children's hospitals. Most older children and adults are immune.

Prevention: Treat at home whenever possible. In hospitals, rapid viral diagnosis, proper isolation procedures and strict adherence to hand washing and gowning procedures are crucial to prevent nosocomial infections. Early discharge from hospital. Vaccine available.

B. Norwalk virus (Now called Norovirus!!!)

Causes outbreaks of gastroenteritis in older children and adults, in any season.

Diagnosis: is important in nursing homes, hospitals, etc, in order to differentiate it from bacterial gastroenteritis. Diagnosis is often arrived at by first excluding a bacterial cause.

Epidemiology: similar to rotavirus, and just as contagious. Fecal-oral route of transmission, but food-borne outbreaks reported from water, shell-fish, etc. Acquisition of immunity much slower than for rotavirus, hence disease in older age groups.

Prevention: same as for rotavirus; however no vaccine. Problem of isolation and cohorting much more difficult
than for rotavirus and complicated by the presence of cases among staff.

C. **Other viruses causing gastroenteritis.**

1. Adenoviruses: some types of adenoviruses can cause gastroenteritis in young children. In contrast to those types causing respiratory disease, the types causing gastroenteritis cannot be cultivated in cell cultures. They are diagnosed by electron microscopy and by other rapid methods.

2. Calici- and astroviruses: these are poorly characterized viruses causing sporadic gastroenteritis in infants and children. Diagnosed by electron microscopy. Transmission by the fecal-oral route.

Summary of viruses causing gastroenteritis

(incubation period: 1- 2 days, for most of them)

	rotavirus	Norovirus	Adenovirus	Calici/astro-virus
Epidemiology	winter epidemics	family and community outbreaks	sporadic	sporadic
age	infants and <2 yr old	older children and adults	infants and children	infants and children
Transmission	fecal-oral	fecal-oral, foods	fecal-oral	fecal-oral
Diagnosis in stools	Electron mic. + imm. methods	Electron mic. + imm. methods	Electron mic. +imm. methods	Electron mic.
Vaccines	yes	none	none	none

Practical importance of rapid laboratory diagnosis: rotavirus and Norovirus extremely contagious! Isolate and/or cohort as soon as possible! But laboratory not useful for "clearing out" patients (in contrast to bacterial gastros). That decision is a clinical one.

4. VIRUS CAUSING EXANTHEMS (RASHES)

A number of totally different viruses are discussed in this chapter. They share common epidemiological features: 1) humans are the sole reservoir; 2) they are highly contagious.

l. Measles

Has probably the highest infectivity rate among viral diseases (i.e., close to l00% of susceptible people in contact with the virus will contract the disease).

Clinical:

- nasal and ocular catarrh (redness) preceding the rash; high fever, Koplik spots.
- rash appears first behind the ears, forehead and around the nostrils - and then spreads to cover the whole body giving a characteristic blotchy appearance.
- the natural disease produces lifelong immunity.

Complications:

- in small children - often secondary bacterial infections e.g., broncho-pneumonia, otitis.
- rarely, encephalitis or subacute sclerosing panencephalitis (SSPE).
- exacerbation of tuberculosis and leukemias.

Laboratory diagnosis very important because: 1) allows to detect the first index case of a potential epidemic; 2) allows to detect which individuals are immune. In both cases, the diagnosis is serological.

1. confirmation of a suspected case: by the demonstration of IgM antibody against measles in a single blood specimen or of a rising antibody titer against measles (IgG) in paired bloods.

2. determination of the immune status of contacts: by the demonstration of circulating measles specific antibody (IgG).

Prevention:

l) Immune globulin: if administered within 5 days of contact with a case of measles, can suppress or attenuate the disease.

2)Live attenuated vaccine: Highly effective and widely used, should be administered after l2 months of age. Most often given as MMR (Measles, Mumps, Rubella vaccine). Revaccination controversial.

2. **Rubella (German measles)**: Mild disease, however extremely dangerous in non- immune pregnant women, because it can produce birth defects in the offspring.

Clinical:

- symptoms similar to measles but milder
- usually enlargement of cervical, retro auricular (behind the ears) and sub-occipital lymph nodes.
- life-long immunity.

In susceptible (non immune) pregnant women, particularly dangerous in the first trimester of pregnancy.

The risk to the fetus:

Rubella during	Risk of damage
0-4 weeks	50%
5-8 weeks	20%
9-l2 weeks	7%

After the third trimester the risk is lower but damage can still be traced up to the 6th month.

Principal birth defects:

Generalized: - abortion, early death of newborn
Localized: - cataract (infection during 6th week)
- deafness (infection during 9th week)
- heart defects (infection during 5th - l0th week)
Others: - low birth weight, cleft palate, dental abnormalities, mental deficiency, etc.

N.B.: In cases of congenital rubella, the virus may persist for up to l8 months (urine) and therefore presents a hazard for susceptible personnel and family contacts.

Epidemiology and Immunity: In the pre-vaccine era, most cases of rubella occurred in school-age children, particularly in the winter and spring, with major epidemics every 7 to l0 years, leading to life-long immunity in 80% of young adults. Now, 60% of cases are seen in those l5 years or older, and may be quite atypical.

Laboratory diagnosis: critical, because atypical cases may spread to susceptible pregnant women or trigger an epidemic. The laboratory confirmation of cases occurring during early pregnancy allows the patient to make an informed decision concerning abortion. The confirmation of a case in a newborn can prevent spread to non-immune nurses who may be pregnant. Finally, the determination of the immune status of contacts is important.

The laboratory diagnosis is serological (several methods).

1. Suspected cases: (acquired or congenital): detection of rubella specific IgM antibody and/or of a rising antibody titer against rubella in paired sera.

2. Immunity status: detection of circulating antibody against rubella (IgG) in a single serum. Several methods have now replaced the old hemagglutination inhibition test (HAI).

Prevention of congenital rubella:

1. Check immune status of women in the child-bearing age: the presence of IgG antibody, by whichever method, indicates immunity .

2. Diagnosis of congenital rubella syndrome in hospitals (risk to non-immune pregnant staff).

3. Rubella serology screening in women and in men starting work in hospitals, clinics, etc.

4. Vaccination of the non-immune with live attenuated vaccines.

5. Strict isolation of congenital rubella cases in hospitals.

Vaccination: with live attenuated vaccine. Vaccination policies:

l. vaccination of babies (combined vaccine MMR) after 12 months
2. vaccination of pre-pubertal girls, if not immune
3. vaccination in the post-partum, if not immune
4. vaccination of hospital personnel, women and men, if not immune

So far, the immunity induced by the live vaccines appears to be long-lasting.

N.B.: Rubella virus has been isolated from the foetus following vaccination of pregnant women. For this reason they should not be given rubella vaccine.

3. **Varicella** (chickenpox)

This is caused by the Varicella/Zoster Virus (VZV), a member of the herpes group.

Clinical:

- Mild childhood febrile illness with a characteristic vesicular rash.

- Successive crops of fresh vesicles appear within 3-4 days after the onset of the rash. Papules, vesicles and crusts are present at the same time.

Complications:

In non-immune adults, occasional pneumonia; may be fatal. Chickenpox can be transmitted to the newborn, if infection occurs close to end of pregnancy.

Herpes Zoster (Shingles) is a limited rash, along the traject of one nerve (often intercostal), accompanied by pain. This form of disease is found mainly in adults and represents a late recurrence of latent varicella infection in a partially immune individual. Cases of zoster may be at the origin of outbreaks of varicella.

Diagnosis: identification of viral particles in vesicles or pustules by electron microscopy or immunological methods, followed by cell cultures.

Prevention:

- vaccine now available
- detection of susceptible individuals in hospital personnel by serological methods
- in specific cases, passive immunization with zoster immuno-globulins (ZIG), or treatment with antivirals

4. **Herpes simplex (HSV)**

One of the most widely disseminated infections. Most infections occur in early childhood,most often inapparently. After the initial infection the virus may persist as a latent infection with reappearance of lesions (clusters of vesicles) "cold sores", triggered by such factors as fever, exposure to sun, etc.

The spread of the infection is difficult to control because of the high percent of inapparent infections, and because it can be transmitted sexually.

Epidemiology

Type l (HSV1) - primarily associated with oral and ocular lesions and transmitted through oral and respiratory secretions: " cold sores"

Type 2 (HSV2) - ("herpes genitalis") - associated with the genital tract and transmitted mainly venereally. The infection in the female can be transmitted to the newborn. Type l can also be transmitted sexually. Possibly some protection afforded by previous Type 1 infection.

Recurrent herpes: occurs with both types, usually in the same region s the primary lesion.

Diagnosis:

1. Identification of virus particles by electron microscopy or immunological methods in clinical specimens
2. The virus grows easily in cell cultures (usually within 24 - 48 hours)
3. Serology of very little use

Clinical forms (apart from cold sores)

- **genital infections:** primary infections and recurrences in both sexes most troublesome
- **herpetic encephalitis**: rare but very serious: see viruses affecting the central nervous system
- **neonatal herpes**: is usually acquired during the passage in the birth canal from an asymptomatic mother infected with either type 1 or type 2. Difficult to prevent. Severity varies from a few vesicles to a fulminant generalized infection, with death or severe sequelae. See Viruses affecting the central nervous system.

- **herpetic whitlow**: herpetic infection affecting the fingers, occupational hazard of health care personnel: can lead to nosocomial infections of newborns in nurseries
- **corneal and conjunctival infectio**n can lead to ulceration of the cornea and blindness
- **relationship to carcinoma of the cervix**: unproven

Treatment/Prevention: antivirals (acyclovir). C-section. No vaccine. No easy way of sorting out pregnant women at risk of transmitting the virus to the newborn.

5. **Papilloma viruses**

These viruses cause different types of warts:

- **common warts** on hands and feet in childhood and adolescence
- **genital warts**: transmission of the genital warts is sexual, but there are asymptomatic carriers, both males and females
- certain types of papilloma viruses definitely associated with anogenital cancer (cervix, vulva, penis) and laryngeal papilloma.

Diagnosis by PAP smears, supplemented by immunological techniques and DNA hybridization methods. No cell cultures available; vaccine now available.

5. VIRUSES CAUSING GLANDULAR ENLARGEMENT

1. Mumps

Like measles, rubella and chickenpox, a contagious disease of childhood caused by a virus. Acute inflammation of the parotid glands. The swelling is most often bilateral. Many inapparent infections also occur.

Complications:

Meningitis: Most frequent form of "aseptic" meningitis in winter months (see viruses affecting the central nervous system)

Orchitis:Inflammation of the testes in about 20% of mumps cases in post-pubertal males. If bilateral, may lead to sterility.

Oophoritis (ovaritis): In about 5% of women with mumps.

Epidemiology: The virus is transmitted by saliva and respiratory secretions. Incubation period is l8-21 days.

Prevention: A live, attenuated vaccine is available (MMR).

2. **Infectious mononucleosis** (glandular fever or kissing disease) (EBV)

Clinical:

Usually mild disease, most often in children or young adults. May sometimes be prolonged and debilitating. Transmission by saliva and droplets.

Characterised by:

Lymphadenopathy, fever, sore throat, lymphocytosis with atypical lymphocytes, often enlargement of liver and spleen.

The Virus:

Epstein-Barr virus - belongs to the herpesvirus family. Like all herpes viruses, establishes a latent infection and may reactivate to cause either chronic disease (rare) or asymptomatic shedding (common) for lifetime of host.

Diagnosis:

a) Blood picture (increase in atypical lymphocytes)

b) Monospot test, detects RBC agglutination, based on heterophile antibody response in which EBV induces the production of a wide range of antibodies, including one that acts as a hemagglutinin

c) Demonstration of the presence of EBV antigens as confirmation

No vaccine available.

3. **Cytomegalovirus infections** (CMV)

Caused by a virus belonging to the herpesvirus group. Mostly symptomless and latent, in the normal individual. But:

Infection during pregnancy, may lead to neonatal infection with jaundice, enlarged liver and spleen, and often mental retardation with microcephaly (reduced head volume) and motor disorders (cerebral palsy).

Disseminated infections in transplanted patients (kidney, heart, bone marrow etc.) can be quite severe and cause rejection of the transplant.

Disseminated infections in AIDS or other immunocompromised patients are frequent and often severe, in particular retinitis and G.I. tract ulcerations.

Diagnosis:

- virus isolation from urines, blood, organ biopsies, etc. is a slow process! But does give definitive results and is faster in immunocompromised patients who have high virus titers.
- CMV antigen detection, DNA hybridization and PCR in leucocytes of the above specimens, are much faster.
- main use of serology is for the screening of donors and recipients before transplant (see below)

Treatment:

- antivirals (ganciclovir and foscarnet)

Prevention (in the immunocompromised host)

- matching the CMV immune status of the donor to that of the recipient, i.e., avoiding if at all possible, transplanting an organ from a seropositive donor into a seronegative recipient
- preventive administration of antivirals
- healthcare workers (seronegative pregnant nurses): the consensus is that universal precautions will prevent transmission

- no vaccine available

6. HEPATITIS VIRUSES

Hepatitis means inflammation of the liver, usually accompanied by malaise, fatigue, nausea, loss of appetite and jaundice. It is caused by hepatitis viruses. The most well known and well characterized are Hepatitis A and B, three others have been described (C, E, G, sometimes called non-A non-B) and there are still cases caused by undefined agents. These are different viruses (not just serotypes) with different transmission patterns and rates of complications.

Apart from the above viruses, other viruses can also cause hepatitis, but as a complication: Epstein-Barr virus, cytomegalovirus, varicella-zoster, and yellow fever virus. Several bacteria can also cause hepatitis. The diagnosis of viral hepatitis is serological, i.e., through the detection of antibodies or of the viral antigens in the peripheral blood. Since the clinical picture is rarely typical, several of the diagnostic tests described below may be carried out simultaneously for a rapid diagnosis.

1. Hepatitis A

Occurs as sporadic cases or as small epidemics, mainly in children or young adults.

Epidemiology: incubation 15-50 days. Transmission by the faecal-oral route. Abrupt onset of disease with acute symptoms. Stools are infectious 2-3 weeks before onset. Infections in children are mild or subclinical. Immunity life-long, no carrier-state, not chronic hepatitis

Diagnosis

- in the suspected clinical case, detection of IgM antibodies.
- detection of IgG antibodies only useful for the detection of immunity, for instance before travel abroad.

Prevention:

- commercial gammaglobulins effective in preventing hepatitis shortly after exposure (family contacts) or for prophylaxis
- vaccine now available for high risk populations

2. **Hepatitis B**

Occurs most frequently as sporadic cases, at all ages.

Epidemiology: incubation average 90 days (7-160). Transmitted most commonly by blood, or blood-contaminated objects, from patients or chronic carriers sharing drugs, needles, toiletries etc. Virus also present in saliva, urine and semen. Sexual and perinatal transmissions also possible. Serum is infective 30-60 days before onset and remains infective for several weeks or months or years (carriers). Minute amounts (mini-droplets) of blood or serum are infective for health care personnel.

Clinically more insidious onset and more severe than hepatitis A, can lead to chronic hepatitis and to the chronic-carrier state.

Diagnosis is based on the appearance in the blood of the hepatitis B surface antigen (HBsAg). The antigen is part of the virus coat. It disappears from the blood within a few weeks or months after onset of jaundice, but, in 5-10 % of cases persist for prolonged periods of time (years or life-long). Such carriers are a source of infective hepatitis B virus.

Antibodies (anti-HBs) appear several months after onset, but fail to appear in carriers. The presence of antibodies is also used as a marker of infection and of immunity.

Prevention

1. Universal precautions for blood and body fluids

2. Proper handling of needles and sharp instruments : NO RECAPPING

3. Screening of blood-, organ-, and sperm donors and pregnant women for HBs antigen.

4. Vaccines made of purified, inactivated HBs antigen or through genetic engineering are a must for health care personnel and is recommended by Health Canada as a part of the routine vaccination

schedule.

5. Specific hepatitis B immune globulins obtained from individuals with high titer of antibody against HBs are effective in preventing hepatitis B after needle pricks, accidental exposure to blood from cases or carriers etc. Are usually part of combined active-passive immunization in two situations:

    a. newborns from HB positive mothers
    b. unvaccinated exposed health care personnel

6. Policy ref. chronic carriers of HBs antigen: not all carriers show the same degree of infectivity. HBs carriers should not donate blood, nor share objects such as razors, toothbrush, etc. HBs positive health care personnel should not work in high risk-areas such as renal dialysis, newborn nurseries, immunosuppressed patients. Still controversial.

3. **Hepatitis C**

Epidemiology: incubation 14-180 days. Transmitted by blood (sharing needles, transfusion, hemodialysis, organ transplants) and sexually. Initial disease is usually mild and without jaundice but results in chronic hepatitis in many patients.

Diagnosis by a serological screening test.

Prevention follows the same lines as those for hepatitis B, but no vaccine available. Treated with recombinant interferon-α and ribavarin.

4. **Hepatitis delta agent**

Epidemiology: incubation 15-64 days. Transmitted by blood (sharing needles, transfusion, hemodialysis, organ transplants) and sexually. This is a viroid-type agent that cannot replicate without the Hepatitis B virus being present in the same cells. It increases the severity of HBV infections and is thought to be responsible for up to 40% of all fulminant hepatitis.

Diagnosis by a serological screening test.

Prevention Vaccination against Hepatitis B prevents infection with the delta agent, since it can only occur in the presence of HBV. Existing HBV infections should avoid further contact with contaminated blood products or sexual partners to decrease risk of acquiring the delta agent.

5. **Hepatitis E virus**

Epidemiology: incubation 15-50 days. Transmitted by fecal-oral route. Symptoms and course of disease similar to Hepatitis A except that HEV infection has a mortality rate of 20% in pregnant women. This virus is seen infrequently in North America but is endemic to India, Pakistan, Nepal, Burma, North Africa and Mexico. Recently cases have been reported in non-travellers in the Netherlands indicating that it is only a matter of time before this virus spreads throughout the world.

6. **Hepatitis G virus**

Epidemiology: incubation 14-180 days. Transmitted by blood and sexually. Initial disease is usually mild and without jaundice but results in chronic hepatitis in many patients.
Diagnosis by detection of viral genome using PCR or other molecular methods.
Prevention as for hepatitis B; no vaccine available.

7. **Yellow fever virus**

Is endemic in Africa, South America and the Caribbean. Transmitted by a mosquito, it causes haemorrhagic fever with hepatitis. Mortality rate in epidemics as high as 50%. Prevention among travellers to some of those countries requires vaccination with the live attenuated yellow fever vaccine.

7. VIRUSES INFECTING THE CENTRAL NERVOUS SYSTEM

A. Clinical manifestations:

1. Aseptic meningitis: describes a syndrome of meningeal inflammation associated with an increase of lymphocytes and other mononuclear cells in the CSF and an absence of cultivable bacteria or fungi.

   The primary site of inflammation is in the meninges without involvement of the neural tissue. Such patients have fever, headache, a stiff neck or back, nausea, vomiting, irritability; their consciousness is intact.

2. Encephalitis: term reserved for patients who show objective signs of CNS dysfunction, such as seizures, paralysis, altered consciousness, etc. This may or may not be associated with aseptic meningitis.

3. Meningo-encephalitis: describes patients with both meningeal and encephalitic manifestations, as described above.

4. Poliomyelitis: refers to the selective destruction of the anterior horn motor cells in the spinal cord and/or the brain, leading to weakness or complete paralysis of muscle groups and, occasionally, respiratory insufficiency. Usually associated with meningitis.

5. Slow virus infections: slow but progressive and persistent viral infections of the CNS, leading to severe and irreversible damage.

B. General diagnostic procedures.

Golden rule: exclude by every possible means the presence of a bacterial or fungal agent!

1. Lumbar puncture: a must in all suspected CNS infections, unless there is increased intracranial

pressure. Four tubes of cerebrospinal fluid (CSF) are needed (0.5 ml each) for:

- CSF biochemistry (cells, proteins and glucose)
- CSF direct Gram stain and cultures for bacteria and fungi
- CSF for detection of bacterial and fungal antigens
- CSF for viral cultures

2. Other specimens: blood for blood cultures, urines for antigen detection, naso-pharyngeal aspirates (Auger suctions), throat swabs, stools or rectal swabs for viral cultures, acute and convalescent sera for viral serology.

C. Viruses with a human reservoir affecting the CNS.

Those viruses involve the CNS as the result of an extension of the primary infection, usually involving another organ or another system.

1. Mumps: most frequent cause of aseptic meningitis in children during the winter months and spring.

2. Enteroviruses (Coxsackie A, Coxsackie B, ECHO, polioviruses): most frequent cause of aseptic meningitis in infants and children during summer and fall.

3. Herpes simplex type 1: a very rare cause of herpetic encephalitis in the young adult, as a result of a latent herpetic infection since the primary infection in childhood.

4. Herpes simplex type 2 or type 1: a very rare cause of meningo-encephalitis in the neonate or the young adult, following neonatal herpes or genital herpes in the young adult.

5. Other human viruses can, very rarely, cause CNS infection as a complication of the primary disease.

Prevention by vaccination? This at present only possible for mumps, measles and poliomyelitis.

D. Viruses with an animal reservoir affecting the CNS

These are rare, because man is only an accidental and dead-end host (i.e., the disease is not transmissible from person to person).

1. Arboviruses: (AR-thropod BO-rne) a complex group consisting of about 200 different viruses, most prevalent in the tropical rain forest areas. The reservoir is in wild animals and the viruses are transmitted to man through an insect bite.

The main disease produced by these viruses is an encephalitis.

In North America, there have classically been four major arboviruses causing encephalitis, Western Equine Encephalitis, Eastern Equine Encephalitis, St. Louis Encephalitis and La Crosse Virus. Recently, the West Nile Virus (named after the Ugandan province where it was first isolated) has been spreading through the US and Canada. This virus normally infects birds, but can be transmitted to humans through a mosquito intermediary. It is not known how the virus was introduced into North America, but it has become a significant public health concern, prompting mosquito control programs. Eliminating these vectors (and their breeding grounds- shallow standing water) is the easiest means of preventing viral spread. There are vaccines available for Western Equine Encephalitis and Eastern Equine Encephalitis but not for the others.

2. Rabies virus: causes an acute encephalitis, always fatal. It affects all mammals and is transmitted and maintained in nature through infected secretions, mainly bites and saliva. In Canada most frequently infected (rabid) animals in wild life are skunks and foxes, which transmit the disease to dogs, cats, cattle, horses and, rarely, man. In man, the disease has a long incubation period, the length depending on the site of bite (average: leg-60 days, arm-40 days, head-30 days). The long incubation period makes possible the prevention of the disease by the use of combined active-passive immunization, a highly effective procedure in exposed individuals.

Post-exposure management (after bites, licks)

1. Wash and disinfect the wound (use a QUAT disinfectant).

2. Capture the animal, if possible, send head to rabies laboratory, for confirmation of rabies.

3. Hyperimmune rabies immunoglobulin, of human origin, immediately.

4. Vaccine - a human diploid cell killed rabies virus vaccine (HDVC) now available; only 5 injections necessary; few side effects.

5. Notify Public Health Officers (Regional Health Units).

Note that several factors have to be considered before embarking in passive -active immunization: species of biting animal, type of biting incident, previous immunization status of the biting animal (if a domestic animal), type of exposure, immunization status of the exposed person, etc.

Prevention (pre-exposure)

1. Vaccinate cats, dogs, horses

2. Vaccinate wildlife (baits with vaccine dropped from air)

3. Vaccinate exposed individuals (veterinarians, pet-handlers, animal handlers, lab. technicians, etc.) with human diploid cell killed vaccine. Check antibody titers annually and boost if necessary.

8. AIDS (ACQUIRED IMMUNODEFICIENCY SYNDROME) AND HIV (HUMAN IMMUNODEFICIENCY VIRUS)

A severe immunosuppressive condition that is often fatal because it predisposes the host to numerous opportunistic infections and cancers. This susceptibility is due to the marked depletion of helper T cells caused by infection with a virus known as HIV (Human Immunodeficiency Virus).

The virus undergoes antigenic changes very easily. Can be grown with some difficulty in lymphocyte cultures, in vitro.

Inactivation: this virus is not particularly resistant to disinfectants per se, but the risk lies in the fact that the virus is often within cells (such as blood cells), which will protect the virus from the action of disinfectants.

It is inactivated by treatment for l0 min. with l0% household bleach, 2% glutaraldehyde (instruments), 6 % hydrogen peroxide. Washing contaminated cloth with detergent and hot water is sufficient to inactivate the virus.

Transmission: sexual, blood and blood derivatives, congenital, organ transplants and sperm donations, drug abusers, etc. The risk of transmission to health care personnel is very small and has been evaluated at less than 0.4%, after cuts or needle pricks with contaminated blood (in contrast to 25-50% risk for hepatitis B). Long asymptomatic period increases the spread of the disease by patients who are unaware they are infected.

Pathogenesis: HIV is cytocidal for T4 helper lymphocytes; neural cells also may be infected. Development of AIDS due to progressive impairment of immunological competence.

Clinical: Following infection, after a long incubation period (6 months - several years), Aids-Related Complex (ARC) disease with characteristic symptoms develops; may or may not progress to full AIDS with depletion of T helper cells and frequent opportunistic infections. In terminal stages of AIDS, most patients develop dementias and other neurological problems, as well as numerous opportunistic

infections (viral, bacterial and fungal).

Laboratory Diagnosis: based mainly on serology. Seropositivity may take up to 6 months to develop after infection. The virus can be isolated from T-cells (blood), plasma, semen, cervical and vaginal secretions, etc. either immediately after infection or late in disease progression.

Prevention: Vaccine development a key focus of research, nothing yet. Therefore must aim to: I) identify all carriers and ii) prevent transmission. Appropriate measures have been established by Federal and Provincial authorities and are similar to those developed to control hepatitis B.

1. universal precautions for health care personnel (see Nosocomial infections)
2. blood, organ and sperm donors must all be screened for HIV
3. heat inactivation of plasma pools for the preparation of factors VIII and IX for the treatment of hemophilias
4. prevention of sexual transmission through sexual education, including use of condoms
5. prevention of transmission through contaminated needles (drug-abusers)
6. prevention of maternal-foetal transmission by testing pregnant women at risk

Treatment: A large number of antiviral drugs exist. Because the virus has a high rate of mutation, the most effective treatment is a combination of drugs from different classes known as Highly Active Antiretroviral Therapy (HAART). This combines a protease inhibitor (stops virus maturation) and reverse transcriptase inhibitors (stops virus replication). Anti-HIV drugs tend to have significant toxic side effects and are very expensive. Acquiring and distributing antiretrovirals for HIV treatment in developing countries (especially in sub-Saharan Africa, which has the highest worldwide rates of HIV infection) is a huge problem.

NOSOCOMIAL (HOSPITAL - ACQUIRED) INFECTIONS

DEFINITION: Infection acquired by a patient during hospitalization and having its origin in the hospital environment or in a medical procedure.

N.B. The definition excludes infections present or incubating on admission. A nosocomial infection may manifest itself after discharge.

Non-preventable infections are due to causes which escape all reasonable means of prevention e.g., infections in the immunodeficient, surgery in patients with a perforated organ (e.g., accidental/shotgun wounds involving the intestines) etc.

Preventable infections are due to faulty medical or nursing techniques (e.g., breaches in aseptic procedures in surgery/catheterization, improper hand washing etc.)

EPIDEMIOLOGY OF NOSOCOMIAL INFECTIONS

In the transmission of an infection by a microorganism three factors play an essential role:

SOURCE --> ROUTE (WAY) OF TRANSMISSION --> HOST

These three elements constitute the chain of infection.

The source is the environment (place) in which microorganisms multiply and from where they can be disseminated. The source can be:

- an infected (or colonized) individual
- an inanimate environment e.g., non-refrigerated food, a water reservoir of a humidifier or improperly sterilized intravenous solutions, etc.

Insofar as patients are concerned the source can be:

- endogenous i.e., patients own flora
- exogenous i.e., outside the patient

N.B. Patients may become colonized by microorganisms acquired in the hospital environment. Whether colonized or infected, a patient may be a source of infection for other patients.

The route of infection is the way by which the infecting microorganisms leaving the source reach the host.

The route of infection may be:

- contact - direct (e.g., person to person)
- indirect through contaminated objects (fomites) e.g., a wound dressing, blood soiled needles, etc.
- water or food
- air
- vectors (e.g., insects)

The susceptible host is an individual infected with a microorganism carried from the source via one of the routes of transmission. In order to establish a chain of infection the microorganism must be able to exit the source and through one of the routes of transmission enter the host.

The aim of hospital infection control programs is to interrupt the chain of infection by:

1) rendering the source non-infectious or by preventing the microorganism from leaving the source.
2) interfering with the dissemination of infectious agents through the different routes of transmission or
3) preventing the microorganisms from entering the host or rendering the host resistant to infection.

Measures can be taken affecting:

1. **The source:**

- detection/identification of sources
- isolation/precautions (infected individuals)
- treatment of infections
- elimination of inanimate sources or pests

2. **The route of transmission:**

- sterilization
- disinfection
- proper medical/nursing techniques
- ventilation
- adequate housekeeping procedures

3. **The host:**

- asepsis (e.g., in surgery)

- vaccination (when possible)

- protective isolation (e.g., isolation of transplant patients)

N.B. Both patients and hospital personnel can be, in turn, source and host i.e., a host once infected may become the source of infection for another individual.

The most efficient step in controlling nosocomial infections is the **identification/detection of the source of infection** followed by measures to prevent the spread of infective microorganisms from the source.

1.1 **Detection of the source** (infected patients) is done by:

- daily review of admissions for identification of patients with infections
- daily review of microbiology reports followed by investigation of suspected cases
- examination of patients' charts and discussions with ward personnel
- special infection report forms filled by ward nurses or physicians

N.B. This is not a complete list; it includes the main methods of case-finding. Other methods like lists of prescribed antibiotics, examination of fever charts, etc. can also be used.

1.2 **Universal precautions and isolation procedures** are aimed at preventing the spread of microorganisms to other patients (and to health care personnel as well!) from the source of infection. They consist of two sets of measures.

1.2.1 **Prevention of transmission through universal precautions** These precautions apply to

apply to all patients without distinction, whether isolated or not, whether treated for infection or not.

1. wearing of gloves for manipulating blood and body secretions
2. hand-washing after each patient
3. no recapping of needles
4. proper handling and disposal of sharp instruments
5. proper and immediate use of disinfectants for contaminated surfaces and antiseptics for contaminated hands and skin
6. wearing of masks and goggles (if risk of splashing)

N.B. Please note that universal precautions are NOT a substitute for isolation procedures.

1.2.2 **Isolation procedures applied to infected patients:** they should be adapted to the mode of transmission of the infecting agent and to the seriousness of the infection. One of two different systems can be used.

1.2.2.1

- disease-specific, in which specific recommendations are made for each of more than 160 different infectious diseases

1.2.2.2

- category-specific, in which diseases with similar degree of danger, route of transmission and similar isolation precautions are grouped together.

2. - **Interference with the route of transmission** is the next step and can be taken independently of the detection of sources of infection.

The aim of these measures is to interrupt the mechanisms of transmission so that microorganisms that might have escaped from the source are prevented from reaching the host. This is done by:

- destroying the organisms deposited on surfaces or penetrating into different objects, by the use of disinfectants or by sterilization.
- eliminating the organisms deposited on surfaces or suspended in the air as aerosols by housekeeping and/or ventilation (air exhaust)
- strict adherence to safe practices/techniques when handling patients

3. - **<u>Protecting the host by</u>**:

- preventing the microorganisms from reaching the host; this is achieved by creating a barrier around the patients e.g., aseptic techniques in surgery or protective isolation of immunodeficient patients.
- immunizing the patient when indicated and possible.

INFECTION CONTROL PROGRAMS

Infection control is, in principle, everyone's business. By their professional standards hospital workers must ensure that patients get the best care with a minimum of risks. In order to back up these efforts, hospitals have organized Infection Control Services which usually include:

- an Infection Control Nurse or Infection Control Practitioner
- an Infection Control Officer/Physician
- an Infection Control Laboratory (or access to laboratory facilities when required)

This group:

- gathers information and analyses data relative to nosocomial infections
- elaborates policies for the Infection Control Committee
- watches the implementation of such policies
- participates in educational programs aiming at familiarization of hospital personnel of all levels with problems of nosocomial infections
- reviews and updates periodically the techniques which may be involved in transmission of infection
- elaborates a "Manual of Procedures in Infection Control" and keeps those procedures up to date.

Each hospital is required by accreditation regulations to have an Infection Control Committee which includes representatives from different hospital sectors (physicians, nurses, administrators, housekeepers, etc.). The Committee analyses the data submitted by the Infection Control Service, debates the issues and recommends to hospital authorities the measures necessary to prevent and control the nosocomial infections.

CLEANING, STERILIZATION AND DISINFECTION

SELECTED GENERAL TERMS

Decontamination: A general term for destroying or removing harmful microorganisms, chemicals or radioactivity on an object or surface.

Sanitization: Reduction of microbial load on objects and environmental surfaces through washing or wiping with detergents or cleaning agents.

Disinfection: Freeing an object of harmful microorganisms, but not necessarily spores. Chemicals used for this purpose are also called **germicides**.

Antisepsis: Use of a germicide on the surface of living skin or mucous membrane.

Sterilization: A physical or chemical means to totally destroy all types of microorganisms in or on an object.

- Killing of at least one million bacterial spores is used as an index of sterility.

CLEANING

- Soil and dirt can shield pathogens from agents used for sterilization or disinfection.
- Therefore, proper cleaning of objects and instruments, such as endoscopes, very important prior to decontamination.

- This step is potentially dangerous to health-care personnel, care must be exercised during pre-cleaning to avoid contact with pathogens.

PHYSICAL MEANS

Methods available: (1) Heat, (2) Radiation and (3) Filtration

(1) HEAT TREATMENT

- Most reliable & common methods when dealing with inanimate objects.
- Variations in resistance of microorganisms and of their spores.
- **Dry heat**: Destruction through oxidation; requires higher temp. and longer times.
- **Moist heat**: Irreversible denaturation of proteins by coagulation (Table 1).

Hot Air Ovens*:* Simple in principle and operation.

- Dry heat non-corrosive, but slow in diffusion and penetration.
- Fan prevents temperature stratification.

At 160°C, minimum time is 60 min.

At 170°C, minimum time is 40 min.

At 180°C, minimum time is 20 min.

Table 1.

Moisture content and coagulation of egg albumin by heat.

Moisture content	Coagulation temperature
50%	56°C
25%	74-80°C
0%	160-170°C

***Incineration**:* Required before safe disposal of waste.

- Temperatures as high as 1000°C.
- Reduction to 10% of original amount.
- Problems of design, operation & image.
- Pathogens in smoke after improper use.
- Transportation of infectious waste.
- Waste should not contain more than 20% plastics.

MOIST HEAT

***Pasteurization**:* Heating below boiling point to destroy vegetative form of pathogens.

- Heat-sensitive liquids & medical devices.
- 62.8°C for 30 min. or 71.7°C for 15 sec. followed by rapid cooling to 10°C.
- Heating sensitive liquids to 80°C for 30 min. on 3 successive days

***Boiling**:* At 100°C for at least 10 min. to disinfect liquids and other objects; this temperature and time not enough to kill spores.

- Sterilization requires longer periods.

- Sea level and boiling temperature.

Autoclaving*:* Steam under pressure

- Most commonly used method.

- Removal of all air important.

- Steam must be 'saturated' (vapor) and 'dry' (no droplets).

- 'Wet' steam soaks into fabrics, impedes heat transfer & prolongs drying time.

- Methods for checking performance of autoclaves

Microwaves: Disinfection of liquids and moisture-containing solid wastes by heating the water in them. May also be used for the sterilization of liquids.

(2) RADIATION

Gamma radiation: Has strong penetrating power and destroys microorganisms mostly through oxidation and damage to DNA.

- It is used to treat certain types of tissues before transplantation and sterilize hospital supplies such as syringes, needles, sutures and plastic containers.

- Highly specialized and expensive facilities are required; not available in most hospitals.

Ultraviolet (UV) rays: UV rays with a wavelength of 260 nanometers (nm) can be germicidal through damage to nucleic acids. Direct and prolonged exposure necessary.

(3) FILTRATION

Physical retention of bacteria and fungi when a fluid is passed through membranes with a pore diameter of 0.2 µm. Commonly used to treat liquids which can be damaged by heat.

CHEMICAL MEANS

- Many types of chemicals are routinely used in health-care settings to prevent and control the spread of infections.
- It is often the responsibility of nurses to know what products to purchase and how to use them properly.
- Product labels are often confusing and claims of product efficacy may be exaggerated.

FACTORS AFFECTING DISINFECTANT ACTION

A clear understanding of the following factors is important for the safe and effective use of chemical disinfectants in health-care settings:

A. *Concentration of germicide:* Higher the concentration, the more rapid the kill

- Use concentration limited by cost, toxicity, corrosiveness and environmental concerns

- Potentiating or synergistic action with other components

B. *Microbes present:* Class and state of microorganism (Figure 1)

- Spore versus vegetative cell; bacteria versus viruses

C. *Contact time:* Most products produce effect within the first few seconds

- Prolonged contact possible only in instrument soaks

D. *Temperature:* Higher the temperature the more rapid the kill

- Enhanced evaporation of certain germicides

E. *Organic and inorganic load:* Protection of microorganism by organic matrix

- Neutralization of germicidal activity by organics

- Water hardness reduces germicidal action

- Precleaning of surfaces and instruments

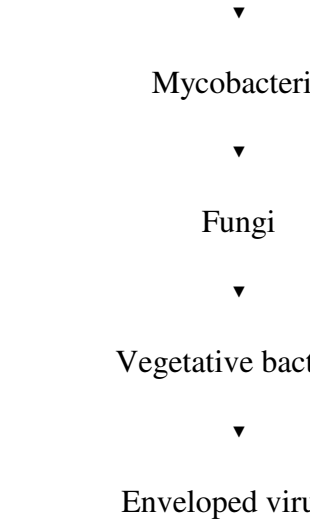
F. *Other factors:* Surface topography

- pH, age, conditions of storage, relative humidity

- Method of application

Figure 1.

The decreasing order of resistance of classes of microorganisms to disinfectants



ANTISEPTICS AND HYGIENIC HAND-WASHING AGENTS

Antiseptics are regularly used as:

(1) *pre-surgical hand scrubs:* Proper use of pre-surgical scrubs with brushing should inactivate and remove all of the transient flora and most of the resident flora.

(2) *pre-operative skin preparations on patients:* Pre-operative skin preps are designed to inactivate the transient as well as the resident flora *in situ*.

(3) *treatment or prevention of infections on skin or mucous membranes:* May contain antibiotics or other anti-microbials for topical use to prevent the multiplication of

microorganisms.

Hand-washing agents for use by health-care personnel generally inactivate and/or remove the transient flora only. They may consist of only ordinary soap or a combination of chemical disinfectants. The formulation must be safe and non-irritating to the skin even after repeated use.

Waterless hand-washing formulations generally contain 60-70% ethanol and an emollients. They are meant to be rubbed on hands after patient contact without the need for subsequent rinsing in water.

Proper and regular hand-washing in health-care settings must be an integral part of any infection control.