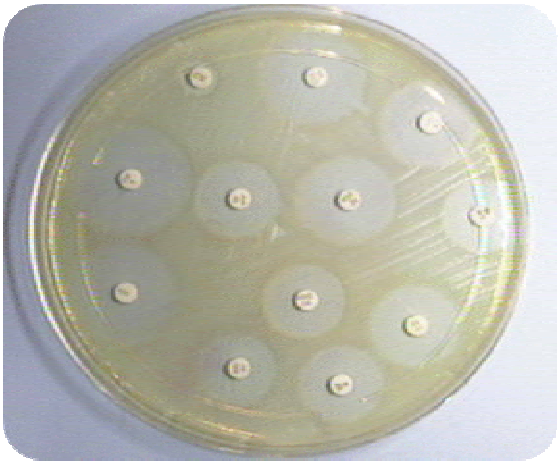
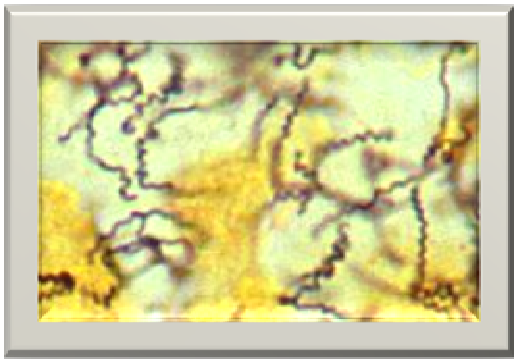
Islamic University-Gaza
Medical Technology Department



Lecture Notes:

Diagnostic Medical Microbiology

MEDI 3313



Dr. Abdelraouf A. Elmanama
Ph. D Microbiology

January 2012

Introduction

- Koch's Postulates
- Basic Definitions
- Factors Controlling Growth of Organisms
- Endotoxin
- Bacterial Exotoxins
- Comparison of Bacterial Exotoxins with Endotoxin
- Summary of Host-Parasite Interactions
- Pathogen Virulence

Koch's Postulates

Four criteria that were established by Robert Koch to identify the causative agent of a particular disease, these include:

1. The pathogen must be **present in all cases of the disease**
2. The pathogen can be isolated from the diseased host and **grown in pure culture**
3. The pathogen from the pure culture must **cause the disease when inoculated into a healthy, susceptible laboratory animal**
4. The pathogen must be **re-isolated** from the new host and **shown to be the same** as the originally inoculated pathogen

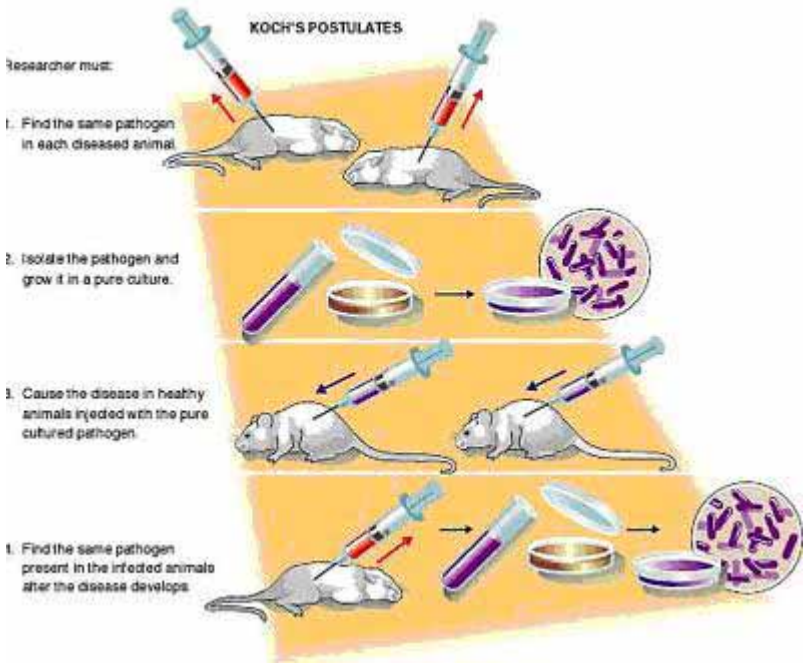


Figure 1: A schematic presentation of Koch's Postulates

Host-Parasite Interaction
Basic Definitions

INDEPENDENCE: *Living free from the influence, guidance, or control by another organism*

SYMBIOSIS: *A relationship in which two dissimilar organisms (SYMBIOTES, SYMBIONTS) live in close association with one another*

MUTUALISM: *Mutually beneficial relationship between two species*

COMMENSALISM: *A relationship between two species in which one is benefited and the other is not affected, neither negatively nor positively*

PARASITISM: *A relationship between two species in which one benefits (parasite) from the other (host); usually involves some detriment to the host*

BENIGN: *Referring to a non-life or non-health threatening condition = COMMENSALISM*

MALIGNANT: *A disease tending to become progressively worse (MORBIDITY=illness) and potentially result in death (MORTALITY=death)*

CARRIER: *A symptomless individual who is host to a pathogenic microorganims and who has the potential to pass the pathogen to others*

PATHOGENICITY: *The quality of producing or the ability to produce pathologic changes or disease*

VIRULENCE: *A measure of pathogenicity; a measurement of the degree of disease producing ability of a microorganism as indicated by the severity of the disease produced; a measure of the dosage required to caused a specific degree of pathogenicity.*

DOSAGE: *The number of pathogenic microorganisms entering the host*

LD50: *The number of microorganisms required to cause lethality in 50% of the test host*

TRUE PATHOGEN: *Any microorganism capable of causing disease; an infecting agent*

OPPORTUNISTIC PATHOGEN: *A usually harmless microorganism that becomes pathogenic under favorable conditions*

INFECTION: *The colonization and/or invasion and multiplication of pathogenic microorganisms in the host with or without the manifestation of disease*

COLONIZATION: *The successful occupation of a new habitat by a species not normally found in this niche*

MULTIPLICATION: *The ability of a microorganism to reproduce during an infection*

DISEASE: *An abnormal condition of body function(s) or structure that is considered to be harmful to the affected individual (host); any deviation from or interruption of the normal structure or function of any part, organ, or system of the body.*

Factors Controlling Growth of Microorganisms

1. NUTRIENT AVAILABILITY: The accessibility of a necessary resource, substance or compound providing nourishment to maintain life, i.e. capable of conversion to energy and structural building blocks

Fastidious: *Having complex nutritional or cultural requirements that make isolation and culture more difficult*
MAJOR ESSENTIAL ELEMENTS:
C, O, H, N, S, P, K, Mg, Ca, Fe, Na, Cl
MINOR ESSENTIAL ELEMENTS:
Zn, Mn, Mo, Se, Co, Cu, Ni, W

2. PHYSICO/ENVIRONMENTAL PARAMETERS:

2.1 WATER ACTIVITY/OSMOTIC PRESSURE:
Water activity (aw): represents the available water
Osmotic pressure: expressed in atmospheres; reflects the concentration of solute in an aqueous solution

2.2 OXYGEN: pathogenic microorganisms may have metabolic oxygen requirements that are: 1. OBLIGATE AEROBES, 2.OBLIGATE 3. FACULTATIVE 4. AEROTOLERANT ANAEROBIC and 5. MICROAEROPHILIC

2.3 pH: "power of hydrogen"; a measurement of the amount of hydrogen ion in solution; the logarithm of the reciprocal of the hydrogen ion concentration in an aqueous solution used to express its acidity or alkalinity (0-14)

2.4 TEMPERATURE:
Psycrophile(psychrophilic): liking cold temperatures; optimal growth at 15^{o} to $20^{o}C$
Mesophile (mesophilic): liking moderate temperatures; optimal growth at 20^{o} to $45^{o}C$
Thermophile (thermophilic): liking elevated temperatures; optimal growth at 50^{o} to $70^{o}C$

3. COMPETITION: the simultaneous demand by two or more organisms or species for a necessary, common resource or physical space that is in limited or potentially limited supply, resulting in a struggle for survival

Niche: the place of an organism within its community or ecosystem

4. HOST IMMUNE SYSTEM: the cells and tissues involved in recognizing and attacking foreign substances in the body

Bacterial Endotoxin

Endotoxin: Complex bacterial toxin; lipopolysaccharide (LPS) component of Gram-negative cell walls is composed of Lipid A + Core Polysaccharide + O Antigen (O polysaccharide side chain) and is released upon lysis of the cell during infection ; Lipid A component is responsible for endotoxin activity effects on the host; O side chain is the antigenic portion of the LPS molecule

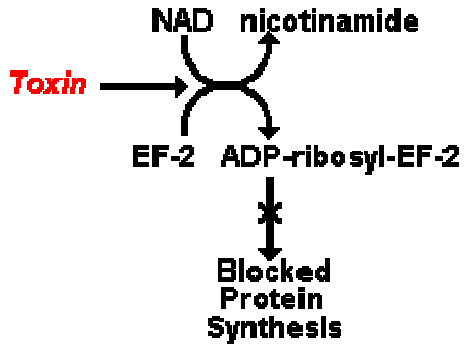
Septic shock (sepsis): Associated with overwhelming infection resulting in vascular system failure with sequestration of large volumes of blood in capillaries and veins; Activation of the complement and kinin systems and the release of histamines, prostaglandins, and other mediators may be involved.
Endotoxemia: Endotoxin in the blood
"More detailed discussion of endotoxin effects can be found later in the text"

Bacterial Exotoxins

Two Broad Classes of Bacterial Exotoxins

1. Intracellular Targets: A-B dimeric (two domain) exotoxins: conform to general structural model (prototype is diphtheria toxin of *Corynebacterium diphtheriae*): Bipartite structure (B, binding; A, active): One component is a binding domain (B) associated with absorption to target cell surface and transfer of active component (A) across cell membrane; once internalized, domain (A) enzymatically disrupts cell function

Receptor-mediated endocytosis (host cell uptake and internalization of exotoxin)
ADP-ribosylation of intracellular target host molecule



2.Cellular Targets: Cytolytic exotoxins (usually degradative enzymes) or cytolysins: hemolysis, tissue necrosis, may be lethal when administered intravenously

Three Major Types of Bacterial Cytolysins Based on Mechanism of Action

1. Hydrolyze membrane phospholipids (phospholipases); e.g., *Clostridium*; *Staphylococcus*
2. Thiol(-SH)-activated cytolysins (oxygen-labile) alter membrane permeability by binding to cholesterol; e.g., streptolysin O of *Streptococcus*; tetanolysin of *Clostridium*
3. Detergent-like activity on cell membranes; rapid rate of lysis; e.g. *Staphylococcus*

Examples of Two-Component (A-B) Exotoxins with Intracellular Targets

1. Adenylate cyclase toxin (*Bordetella* spp.):
- Chromosomally-encoded
- Activated by intracellular calmodulin and, like pertussis toxin, catalyzes conversion of ATP to cAMP
- Inhibits leukocyte chemotaxis and activity

2. Anthrax toxin (*Bacillus anthracis*):
- Plasmid-encoded
- Three separate proteins: Protective antigen (PA); Edema factor (EF); Lethal factor (LF)
- EF + PA = increase in cAMP level resulting in edema (fluid accumulation)
- LF + PA = death of host cells and ultimately death of host

3. Botulinum toxins (7 antigenically distinct toxins (A-G)) (*Clostridium botulinum*):
- Phage-encoded neurotoxins
- Among most potent of all biological toxins
- Binding domain (B-subunit) binds to neuroreceptor gangliosides on cholinergic neurons
- A-subunit irreversibly inhibits release of the stimulatory neurotransmitter, acetylcholine, at myoneural (muscle-nerve) junctions (peripheral cholinergic synapses) resulting in a flaccid paralysis and death

4. Cholera toxin (A-5B) (*Vibrio cholerae*):
- Chromosomally-encoded
- B-subunit binds to GM_1 ganglioside receptors in small intestine
- Reduction of disulfide bond in A-subunit activates A_1 fragment that ADP-ribosylates guanosine triphosphate (GTP)-binding protein (G_s) by transferring ADP-ribose from nicotinamide adenine dinucleotide (NAD); the ADP-ribosylated GTP-binding protein activates adenyl cyclase resulting in an increased cyclic AMP (cAMP) level and a profound life-threatening diarrhea with profuse outpouring of fluids and electrolytes (sodium, potassium, bicarbonate) while blocking the uptake of any further sodium and chloride from the lumen of the small intestine and ultimately resulting in hypovolemic shock and death in the absence of fluid and electrolyte replacement therapy

5. Diphtheria toxin (A-B) (*Corynebacterium diphtheria*):
Phage-encoded
ADP-ribosylation inhibits cell protein synthesis by catalyzing transfer of ADP-ribose from NAD (nicotinimamide adenine nucleotide) to EF-2 (elongation factor - 2)

6. Exotoxin A (*Pseudomonas aeruginosa*):
Chromosomally-encoded
Similar or identical to diphtheria toxin

7. Heat-labile enterotoxins (HLT or LT) (LT-I and LT_II) (enterotoxigenic *Escherichia coli* (ETEC):
LT-I is plasmid-encoded
LT-II only produced by strains isolated from animals
Similar or identical to cholera toxin

8. Heat-stable enterotoxins (STa and STb) (enterotoxigenic *Escherichia coli* (ETEC):
- STa is plasmid-encoded
- STb only produced by strains isolated from animals
- Similar to LT-I and cholera toxin, but with increased levels of cyclic guanosine monophosphate (cGMP) leading to hypersecretion

9. Pertussis toxin (A-5B) (*Bordetella pertussis*):
- Chromosomally-encoded
- S2 (B) subunit binds glycolipid receptor on ciliated respiratory cells; S3 (B) subunit binds to glycolipids on phagocytes
- S1 (A) subunit inhibits signal transduction via ADP-ribosylation of GTP-hydrolyzing protein (G_i) with unregulated adenylate cyclase and increased levels of cAMP resulting in hypersecretion of respiratory secretions and mucus and paroxysmal cough
- Inhibits leukocyte chemotaxis and activity

10. Shiga toxin (A-5B) (*Shigella dysenteriae*):
- Chromosomally-encoded
- Among most potent of all biological toxins
- B-subunit binds to Gb_3 glycolipid receptor
- A-subunit prevents binding of aminoacyl-transfer RNA by cleaving 28S rRNA from 60S ribosomal subunit resulting in inhibition of protein synthesis

11. Shiga-like toxins (A-5B) (SLT-I and SLT-II in EHEC) (enterohemorrhagic *E. coli* (EHEC); *Shigella* spp.):
- Phage-encoded
- SLT-I identical to *S. dysenteriae* Shiga toxin with the exception of a singel amino acid
- SLT-II has ~60% homology with Shiga toxin
- B-subunit binds to target cell glycolipid globotriaosylceramide
- Similarly to cholera toxin A subunit is cleaved; A_1 fragment binds to 28S rRNA of 60S ribodomal subunit and protein synthesis is inhibited

12. Tetanus toxin (*Clostridium tetani*):

- Plasmid-encoded neurotoxin
- Among most potent of all biological toxins released upon lysis of bacterial cell
- Binding domain (B) binds to neuroreceptor gangliosides (GD_{1b})
- A-subunit (zinc endopeptidase) is internalized and migrates from peripheral nerves to central nervous system and across synapses to pre-synaptic nerve endings (retrograde, i.e., against the normal direction of nerve impulses) where it is accumulated in vesicles and irreversibly blocks the release of inhibitory transmitters resulting in continuous stimulation of muscles by excitatory transmitters resulting in spastic paralysis (spasms of bulbar and paraspinal muscles) with trismus (lockjaw; spasms of the masticatory muscles), risus sardonicus (spasms of the masseter muscles) and opisthotonos (spasms of back and neck muscles)

Table 1: Comparison between Exotoxin and Endotoxin

Exotoxin	Endotoxin
Produced by both Gram-positive and Gram-negative bacteria	Produced only by Gram -negative bacteria
Released from cell	Integral part of cell wall
Protein	Lipid A of lipopolysaccharide
Many types of exotoxin based on structure and function	Only one type of endotoxin
Heat labile	Heat stable
Specific receptors on host target cells	Diverse range of host cells and systems affected
Specific effects in host	Diverse range of effects in host
Toxoids can be made by treating with formalin	Toxoids cannot be made

Summary of Host-Parasite Interactions

NATURAL HABITATS FOR MICROBES

- Soil; Water; Air
- Man, Animals and Animal Products

MICROBIAL FLORA (microbiota) OF HUMAN BODY

Skin: Gram-positive bacteria are most common

Respiratory Tract (actually external to body)

Mouth and **oropharynx**

- Saliva
- Teeth and gums (gingiva)

Nose and **nasopharynx**
Microorganisms can be aspirated into the lower respiratory tract

- Larynx, trachea and bronchial tubes (**bronchi**)
- Lungs: Superior and inferior lobes (also middle lobe in right lung); Alveoli

Eye (Conjunctivae)

Ear: Inner, middle and exterior; Exterior commonly colonized

Gastrointestinal Tract (actually external to body):

Intestinal flora play a significant role in: Digestion; Vitamin production (e.g., vitamin K); Ecological competition (see below) with potentially pathogenic microorganisms

- Esophagus: Not typically colonized
- **Gastric mucosa** of stomach: Acid tolerant organisms
- Small intestine: Extends from **pylorus** to **ileo-cecal junction** (about 20 feet in length); Colonized by mostly **anaerobes**

Villi (plural of villus) and **microvilli** are finger-like projections that protrude through the mucous membrane throughout the length of the small intestine and are responsible for absorption
Peyer's patch: Aggregations of **lymphoid tissue** concentrated in the ileum
M (microfold) cells: specialized cells in the Peyer's patches that sample the microenvironment and uptake foreign antigens for processing by underlying **macrophages**
Duodenum: Upper portion of small intestine (about 10 inches in length) encompassing the superior, descending, transverse and ascending portions, in that order; Hepatic ducts (from liver), pancreatic duct, and cystic duct (from gallbladder) join and enter into the intestine at the descending duodenum
Jejunum (middle portion): Upper two-fifths of remaining length of small intestine
Ileum (lower portion): Remaining three-fifths of length of small intestine

- Large intestine: Extends from **ileo-cecal valve** to anus (about 5 feet in length); $>10^{11}$ bacteria per gram of feces with anaerobes 1000-fold more common than other microbes

Cecum (a.k.a., **Caecum**): Large, blind pouch just posterior to the ileo-cecal junction
Colon: Ascending, transverse, descending, and sigmoid portions

- Rectum
- Anus

Genitourinary Tract

1. Anterior urethra: Normally colonized by avirulent organisms; May be transiently colonized by fecal organisms that can cause disease; *Neisseria gonorrhoeae* and *Chlamydia trachomatis* may cause disease or asymptomatically colonize
2. Urinary bladder: Not normally colonized; May be transiently colonized with urethral organisms
3. Vagina: Microbial population influenced by hormones
4. Cervix: Not normally colonized; *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are important pathogens

NORMALLY STERILE SITES IN THE HUMAN BODY

Colonization of one of these sites generally involves a defect or breach in the natural defenses that creates a portal of entry

- Brain; Central nervous system
- Blood; Tissues; Organ systems
- Sinuses; Inner and Middle Ear
- Lower Respiratory Tract: Larynx; Trachea; Bronchioles (bronchi); Lungs; Alveoli
- Kidneys; Ureters; Urinary Bladder; Posterior Urethra
- Uterus; Endometrium (Inner mucous membrane of uterus); Fallopian Tubes; Cervix and Endocervix

ECOLOGY DEFINITIONS

- **Ecological Niche**: Unique environmental position occupied by a particular species, perceived in terms of actual physical space occupied and function performed within the community or **ecosystem**
- **Flora** (Microbiology Definition) = **Microbiota**: Microorganisms present in or characteristic of a special location (**Flora** more generically refers to plants; **Fauna** generically refers to animals)
    - **Normal flora = Indigenous or resident microbiota**: Microbial flora typically occupying a particular niche; Organisms tend to segregate given diversity of environmental conditions; Many normal flora perform important functions for the host, including: digestive and nutritional functions and competition with pathogenic microorganisms
    - **Transient flora**: Microbial flora only temporarily associated with a particular niche
    - **Endogenous flora**: Microbial flora occupying niches that are in or on the body of the host
    - **Exogenous flora**: Microbial flora normally existing externally to the body of the host

ECOLOGICAL RELATIONSHIPS

- **Independence**: Living free from the influence, guidance, or control by another organism
- **Benign** Relationship (**Commensalism**): **Carrier**
- **Malignant** Relationship (**Parasitism**): **Disease**
    - **Benign**: Referring to a non-life or non-health threatening condition; commensalism between host and parasite
    - **Carrier**: Symptomless individual who is host to a pathogenic microorganim and has the potential to pass the pathogen to others
    - **Malignant**: Disease tending to become progressively worse (**Morbidity** = illness) and potentially result in death (**Mortality** = death)
- **Microbial Interactions**: Complex relationships among species; **Neutral**, **Antagonistic**, or **Synergistic**
- **Host-Parasite Interactions**: **Commensalism (+/0); Mutualism (+/+); Parasitism (+/-)**
    - **Symbiosis**: A relationship in which two dissimilar organisms (**Symbiotes**, Symbionts) live in close association with one another
    - **Commensalism**: A relationship between two species in which one is benefited and the other is not affected, neither negatively nor positively

- **Mutualism**: Mutually beneficial relationship between two species
- **Parasitism**: A relationship between two species in which one benefits (**Parasite**) from the other (**Host**); Usually involves some detriment to the host
    - **True pathogen (Strict pathogen)**: Any microorganism capable of causing disease; An infecting agent
    - **Opportunistic pathogen**: A usually harmless microorganism that becomes pathogenic under favorable conditions; Often a member of the normal microbial flora

EPIDEMIOLOGY

Study of factors influencing occurrence, transmission, distribution, prevention and control of disease

- **Epidemic**: Occurring suddenly in numbers clearly in access of normal expectancy
- **Endemic**: Present or usually prevalent in a population or geographic area at all times
- **Pandemic**: Widespread epidemic distributed or occurring widely throughout a region, country, continent, or globally

- **Acquiring Infectious Agents**
    - **Portals** (Routes) **of entry**: Ingestion, inhalation, direct penetration
    - **Carrier state**: Symptomless individual (host) that is colonized by a pathogenic microorganism and who has the potential to pass the pathogen to others; Carriage may be transient or (semi-) permanent
    - **Nosocomial infections**: Infection acquired in a hospital setting that was not present in the host prior to admission, generally occurring within 72 hours of admission
    - **Opportunistic infections**: Infection caused by a normally harmless microorganism when certain predisposing conditions (disease or conditions that increase host susceptibility) exist
- **Transmission of Disease**
    - **Portals of entry/exit**
    - **Vector**: Living carrier, especially the animal that transfers an infectious agent from one host to another; Commonly an **Arthropod**
    - **Fomite**: Inanimate object capable of transmitting microbes from one host to another, e.g., soiled bed linens, diapers, tissues and handkerchiefs, hospital respiratory equipment, etc.

VIRULENCE FACTORS

1. Colonization Factors

1.1 Attachment/Adherence: Close association of bacterial cells and host cells generally characterized by receptors and target sites

1.2 Surface Receptors/Target Sites: Receptor sites present on both host (**Receptor**) and bacterial surfaces (**Adhesins**)

1.3 Adhesins: Bind Specific Host **Receptors**; Often involve fimbriae as structural cell component; Host cell receptors are often sugar moieties; **Lectin**: Adhesin specific for polysaccharide target receptor (sugar residues)

1.3.1 Fimbriae (plural): Modern term for short, hair-like, protein (**pilin**) appendages extending outward from the surface of certain bacteria (formerly and a.k.a., pili)

1.3.2 Pili (plural); **Pilus** (singular): Short, hair-like protein (**pilin**) appendages extending outward from the surface of certain bacteria; Term more properly applied to those organelles (**F-pilus**) responsible for **bacterial conjugation** (transfer of nucleic acids between closely related strains or species = "bacterial sex")

2. Invasive Factors:

2.1 Invasins enable a pathogenic microorganism to enter and spread throughout the cells and/or tissues of the host body; Specific recognition of receptor sites on target cells enhances pathogenic advantage

2.2 Degradative Enzymes: Class of protein capable of catalytic reactions. Bacterial growth requires food and energy: Growth is achieved by enzymatic catalysis of catabolic (breakdown) reactions of host tissues (resulting in tissue damage) linked to catalysis of anabolic (buildup) reactions in the bacterial cell. Bacterial and host enzymes both play roles in the disease process

3. Toxigenicity

The ability of a microorganism to cause disease as determined by the toxin it produces which partly determines its virulence.

3.1 Toxin-like **pyrogenic** (fever inducing) cell components (e.g., peptidoglycan and peptidoglycan fragments; Teichoic acid or lipoteichoic acid of Gram-positive cell wall)

3.2 **Endotoxin**: Complex bacterial toxin; Lipid A portion of lipopolysaccharide from Gram-negative cell walls; LPS is composed of Lipid A + Core Polysaccharide + O Antigen (a.k.a., O polysaccharide side chain) and is released upon lysis of the cell during infection; Lipid A component is responsible for endotoxin activity effects on the host; O side chain is the antigenic portion of the LPS molecule

Effects of endotoxin: Binds to specific receptors on macrophages, B lymphocytes (a.k.a., B cells) and other cells stimulating production and secretion of acute phase immunoreactants and lymphokines (e.g., IFN-gamma, IL-1, TNF-alpha, IL-6, histamine, prostaglandins); Stimulates growth of B cells (mitogenic).

- **Lymphocyte**: Agranular leukocyte that is concentrated in lymphoid tissue and is active in immunological responses in the body, including the production of lymphokines (cytokines) and antibodies

- **Septic shock (sepsis)**: Endotoxemia; Endotoxin in the blood; Associated with overwhelming infection resulting in vascular system failure with sequestration of large volumes of blood in capillaries and veins; Activation of the complement and kinin systems and the release of histamines, prostaglandins, and other mediators may be involved

- **Fever (Pyrogenicity)**: Any elevation of the body temperature above the normal; Functions to speed up immune reactions and to limit/slow bacterial growth and multiplication

- **Leukopenia** and then **Leukocytosis**: Abnormal reduction in the number (-penia) of leukocytes in the blood, (specifically a count of 5000 or less per cubic millimeter) / Abnormal increase in the number (-cytosis) of leukocytes in the blood, as during hemorrhage, infection, inflammation, or fever (specifically a count of 12,000 or more per cubic millimeter), respectively

- Activation of **alternate complement pathway**: C3a; C5a

- Increased vascular permeability (vasodilation); Decreased peripheral circulation;
- Decreased perfusion (blood flow) of blood to major organs

- **Petechiae**: Round, purple lesions caused by intradermal or submucosal microvascular hemorrhaging; capillary leakage; microhemorrhage

- **Hypotension**: Low blood pressure

- Effects on **metabolic and liver functions**

- **Decreased iron**

- **Hypoglycemia**: Abnormally low glucose levels

- Activation of **clotting pathway**

- **Thrombocytopenia**: Abnormally low numbers of blood platelet

- **Thrombosis**: formation of blood clot (thrombus) in heart or blood vessel

- **(DIC) Disseminated intravascular coagulation**: Disorder characterized by a reduction in the elements involved in blood coagulation due to their utilization in widespread blood clotting within the vessels; Late stages marked by profuse hemorrhaging

- **Shock**: Characterized by failure of the circulatory system to maintain adequate blood flow to the vital organs; Symptoms include: Hypotension; Weak pulse; Rapid and shallow breathing; Low body temperature; CNS (central nervous system) effects (e.g., nausea)

- **Death**

3.3 **Exotoxins**: Potent toxic substance formed and secreted extracellularly by species of certain bacteria; Genetic control can be encoded either **chromosomally**, on a **plasmid**, or by a **lysogenic bacteriophage**

*"**Toxoid**: Toxin that can be altered with formaldehyde to lose physiological toxicity while retaining **antigenicity**; used as a **vaccine**"*

3.3.1 Bacterial Cytolysins (**Cytotoxins**; Cytolytic toxins; Cytolytic enzymes): Responsible for **hemolysis** and **tissue necrosis**; May be lethal when administered intravenously

***Three major types** based on mechanism of action:*
3.1.1.1 Hydrolyze membrane phospholipids (e.g., phospho-lipases of *Clostridium, Staphylococcus*)
3.1.1.2 Thiol-activated cytolysins (oxygen-labile) alter membrane permeability by binding to cholesterol; e.g., *Streptococcus, Clostridium*
3.1.1.3 Detergent like activity on cell membranes; e.g. *Staphylococcus*, rapid rate of lysis

3.3.2 Two-Component (Bipartite; Two domain) Toxins (A-B or A-5B): Usually one component is a **receptor-binding domain (B)** associated with absorption to target cell surface and transfer of active component across cell membrane; Second component is an **enzymatic domain (A)** (**active component**) that enzymatically disrupts cell function

Major properties

- Conform to **general structural model**: **Prototype** is diphtheria toxin of *Corynebacterium diphtheriae*
- **Bipartite** structure (B, binding; A, active)
- **Receptor-mediated endocytosis** (host cell uptake and internalization of exotoxin
- **ADP-ribosylation of intracellular target** host molecule (e.g., host EF-2 (elongation factor-2) is ADP-ribosylated by *C. diphtheriae* exotoxin)

3.3.3 Other types of exotoxins (PA, EF, LF protein toxins of *Bacillus anthracis*).

Bacterial Defenses against Host Responses to Infection

4.1 Encapsulation and **antigenic mimicry, antigenic masking,** and **antigenic shift** are important bacterial defense mechanisms

Evasion or incapacitation of phagocytic and/or immune clearance

Phagocytosis inhibitors: Mechanisms enabling an invading microorganism to resist being engulfed, ingested, and or lysed by phagocytes/ phagolysosomes; Patients with a defective/compromised **monocyte-macrophage system** (formerly, RES, **reticuloendothelial system**) are particularly susceptible to infection

4.2 **Capsule** (Slime layer)

4.3 Avoid recognition and killing
4.3.1 Inhibit **opsonization**, **chemotaxis**, and/or **phagocytosis**
4.3.2 Inhibit phagolysosomal fusion and/or resist lysosomal killing
4.3.3 Block activation of phagocytes by **interferon-gamma**
4.3.4 Destroy (lyse) phagocytic cell

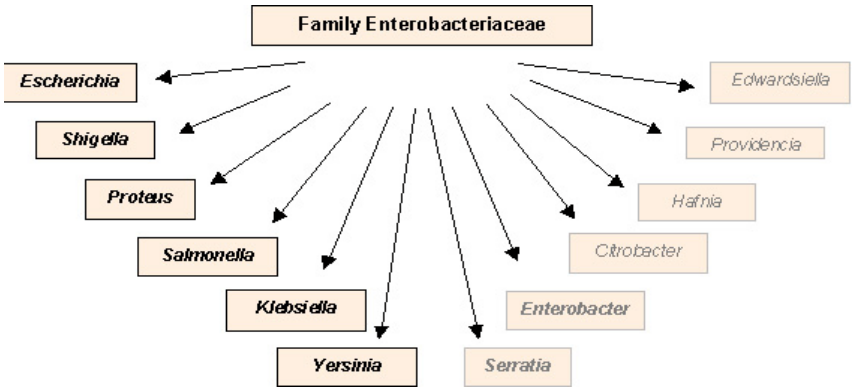
4.4 Inactivate/evade **complement** and **antibody**
4.4.1 Evade alternate complement system
4.4.2 Survive opsonization in presence of complement and PMNs and survive inside phagocytic cells
4.4.3 Avoid antibody or proteolytically cleave **immunoglobulins**

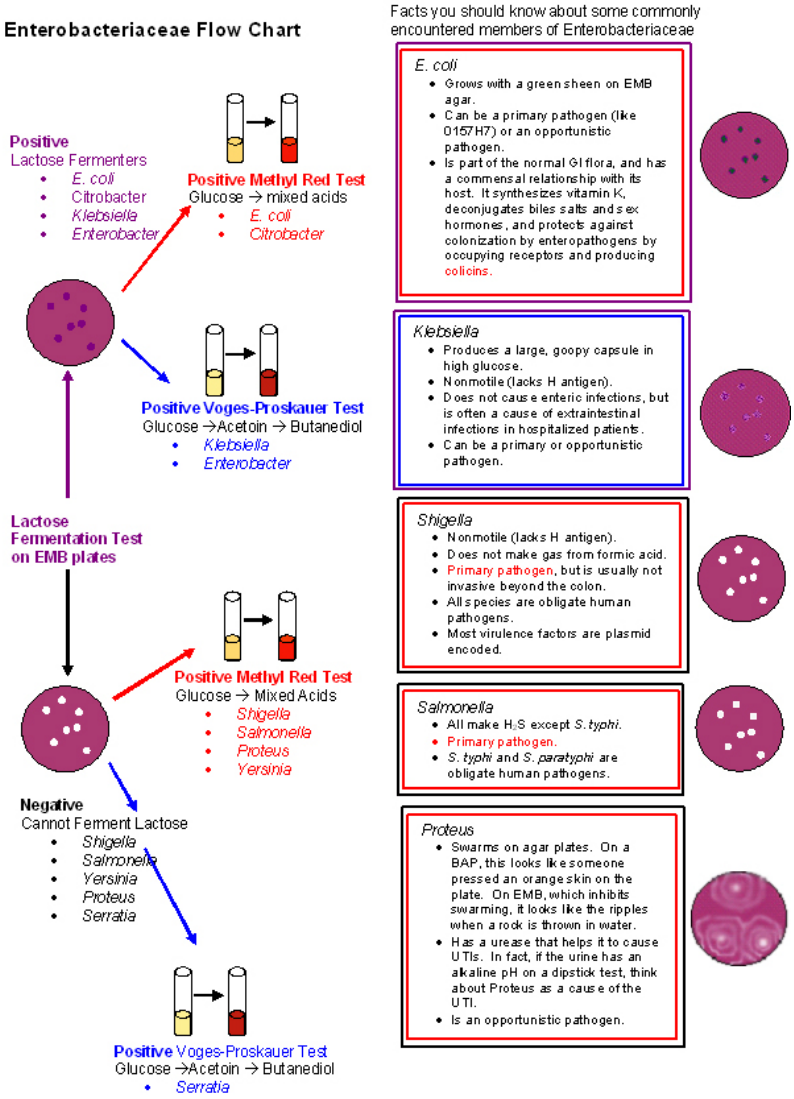
4.5 Avoid immune response by growing intracellularly
4.5.1 Direct invasion of cells
4.5.2 Resist lysosomal enzymes and antibacterial substances and multiply intracellularly
4.5.3 Escape phagosome; Adapt to cytoplasmic growth

4.6 Nonspecific T cell activation and/or mast cell stimulation: Bind TCR on T cell and MHC II on APC without presence of antigen; Life-threatening release of excess interleukins and mediators; Toxin-like, autoimmune-like responses or loss of immunoresponsiveness

4.7 Induction of excess or chronic inflammation; Fibrosis (walling-off) of site of infection (e.g., granuloma formation seen in mycobacterial infections

4.8 Resistance to antibiotics: **Intrinsic resistance**; Plasmid-mediated; Chromosomally-mediated





Facts you should know about some commonly encountered members of Enterobacteriaceae

E. coli
- Grows with a green sheen on EMB agar.
- Can be a primary pathogen (like 0157:H7) or an opportunistic pathogen.
- Is part of the normal GI flora, and has a commensal relationship with its host. It synthesizes vitamin K, deconjugates biles salts and sex hormones, and protects against colonization by enteropathogens by occupying receptors and producing colicins.

Klebsiella
- Produces a large, goopy capsule in high glucose.
- Nonmotile (lacks H antigen).
- Does not cause enteric infections, but is often a cause of extraintestinal infections in hospitalized patients.
- Can be a primary or opportunistic pathogen.

Shigella
- Nonmotile (lacks H antigen).
- Does not make gas from formic acid.
- Primary pathogen, but is usually not invasive beyond the colon.
- All species are obligate human pathogens.
- Most virulence factors are plasmid encoded.

Salmonella
- All make H_2S except *S. typhi*.
- Primary pathogen.
- *S. typhi* and *S. paratyphi* are obligate human pathogens.

Proteus
- Swarms on agar plates. On a BAP, this looks like someone pressed an orange skin on the plate. On EMB, which inhibits swarming, it looks like the ripples when a rock is thrown in water.
- Has a urease that helps it to cause UTIs. In fact, if the urine has an alkaline pH on a dipstick test, think about Proteus as a cause of the UTI.
- Is an opportunistic pathogen.

The Gram Negative Bacilli
Family Enterobacteriaceae

Organisms in this group form one of the largest and well defined groups amongst **Gram (-)** non-photosynthetic bacteria.

All have small, **rod shaped cells**, either straight or curved, not more than 1.5 μm in width.

Facultative aerobes fermenting sugars into a variety of end products. Produce acid from glucose. Ability to ferment glucose, separates them from obligate aerobes.

Some immotile and some motile with **peritrichous flagella**.

Catalase (+) and **oxidase (-).**

Enterobacteriacae are always oxidase (-) hence separates them from oxidase (+) bacteria such as *Pseudomonas*, *Aeromonas*, *Vibrio*, *Flavobacterium*, *Cardiobacterium* species which have similar morphology.

Best known is *Escherichia coli*, characteristic member of normal intestinal flora of mammals and also a important pathogen causing intestinal and urinary tract infections.

Closely related are other Enterobacteriacae such as *Salmonella* and *Shigella* species, pathogens which cause intestinal infections such as dysentery, typhoid fever and food poisonings.

Of different ecology are the genera *Serratia* and *Proteus* which primarily occur in soil and water, and the plant pathogen *Erwina*.

Another member is *Yersinia* species which includes *Yersinia pestis*, the agent of bubonic plague.

All have lipopolysaccharide (LPS) outer membrane, which is responsible for some of the pathogenic symptoms.

Toxic properties are usually associated with part of this LPS layer. This region of LPS is called endotoxin. Cell bound and only released when cells lyse.

The following table indicates the tribes and genera included in the family enterobacteriacae. "this classification is not permanent and subject to continuous changes"

Table 2: Family enterobacteriacae

Tribes	Genera
1. Escherichiaceae	1. Escherichia 2. Edwardsiella 3. Citrobacter 4. Salmonella 5. Shigella
2. Klebsiellae	6. Klebsiella 7. Enterobacter 8. Hafnia 9. Serratia
3. Proteae	10. Proteus 11. Providencia 12. Morganella
4. Yersiniae	13. Yersinia
5. Erwiniae	14. Erwina

This group is further subdivided by lactose fermentation into lactose fermenter and non lactose fermenters. This could be easily done by growing the bacterium on a differential medium "e.g., MacConkey agar plates" wherein lactose fermenting enterobacteriacae produces acid that will change the color of the medium into red while non-lactose fermenters will remain colorless. Some may show delayed lactose fermentation "e.g., after 48 to 72 hours of incubation".

Table 3: Classification of enterobacteriacae according to their ability to ferment lactose

Lactose fermenters (LF)	Non-lactose fermenter (NLF)	Delayed lactose fermenter (DLF)
1. Escherchia	1. Salmonella	1. Morganella
2. Enterobacter	2. Shigella	2. Providencia
3. Klebsiella	3. Proteus	3. Serratia
4. Citrobacter	4. Yersinia	4. Edwardsiella
		5. Erwinia
		6. Hafnia

ANTIGENIC PROPERTIES OF ENTEROBACTERIACAE

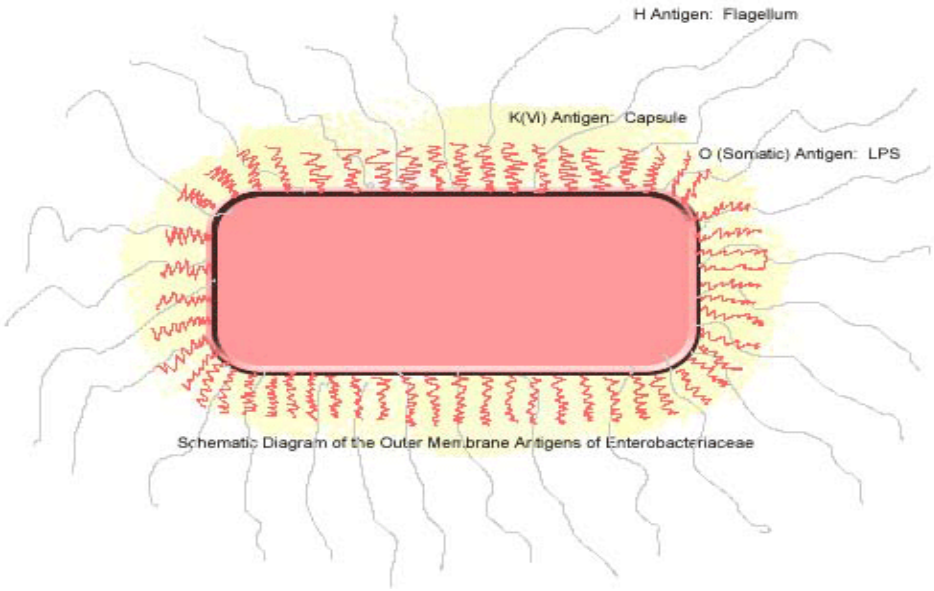
This group of organisms has a complex antigenic structure, and strains are divergent in their serologic behavior.

1. O Antigen: Somatic antigen. Heat stable antigen. O antigens are lipopolysaccharides and are found in the cell wall of most of gram-negative bacilli. With sera containing anti-O antibodies, such antigens agglutinate slowly in granular masses. Antibodies to O antigens are predominantly IgM.

2. H Antigen: Flagellar Antigen: This antigen is heat labile and could be inactivated by heating over 60 ^{o}C. With sera containing anti-H antibodies, such antigens agglutinate rapidly. Within a single Salmonella species, flagellar antigens may occur in either or both of 2 forms, called phase I and phase II. The organism tends to change from one phase to other; this is called phase variation. Antibodies to H antigen are predominantly IgG.

3. The "Vi" Antigen: Capsular (K) antigens that are present at the extreme periphery of the bacteria. Often interfere with agglutination of freshly isolated strains by antisera containing mainly anti-O agglutinins. Vi antigens are destroyed by heating for 1 hour at 60 ^{o}C.

Example of antigenic designation of Salmonella:
Salmonella typhi O 1,2,(Vi): a: 1
= 1,2 are O antigens
= (Vi) if present
= a phase one H antigen.
= 1 phase two H antigen.

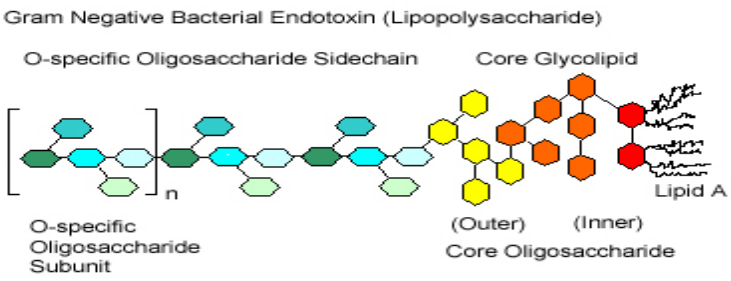


Enterobacteriaceae have the following distinctive characteristics:
1. Gram negative bacilli
2. Facultative anaerobes (grow with or without oxygen).
3. Glucose fermenters.
4. Oxidase negative
5. Nitrate positive

Pathogenic Determinants

1. Endotoxin: It is a lipopolysaccharide in structure and is derived from bacterial cell wall during lysis which may produce the following conditions:

A. Fever
B. Lethal shock
C. Diarrhea
D. Abortion



2. Colicins: Bacteriostacins with antibiotic like substance produced by certain strains of *E. coli* and other related members resulting in the death and lysis of other sensitive cells releasing the endotoxin.

The Cultivation, Isolation and Differentiation of Members of Enterobacteriacae

Media of Choice:

1. Eosin Methylene Blue (EMB)
2. MacConkey Agar (the most commonly used media)
3. Hektoen enteric
4. Endo agar
5. *Kligler`s Iron Agar (KIA)* — *For initial differentiation*
6. *Triple Sugar Iron Agar (TSIA)*

A primary differentiation is obtained by transferring colorless colonies to TSIA slants. Degradation of sugar and accompanying acid production are detected by pH indicator, phenol red which changes its color from red orange to yellow. Thiosulfate is reduced by H_2S which reacts with iron salt to give black iron sulfide.

ESCHERICHIAE

Escherichia coli is a bacterium, which inhabits the intestinal tract of humans and other warm-blooded mammals. It constitutes approximately 0.1% of the total **bacteria** in the adult intestinal tract. Its name comes from the name of the person, Escherich, who in 1885 first isolated and characterized the bacteria.

Species:

Escherichia coli = Most frequently isolated
E. fergusonii
E. hermannii
E. vulneris

E. coli is a normal inhabitant of the intestinal tract and may cause a wide variety of diseases. Infants diarrhea, meningitis, wound infection, urinary tract infection.....etc. The presence of this organism in water is used as an indicator of recent fecal contamination because this organism does not survive in water for long. Strains of *E.coli* which are capable of causing disease, possess one or more virulence factor:

1. Enterotoxigenic *E. coli* (ETEC): Produces a heat-labile toxin (LT) an a heat-stable toxin (ST): LT action is identical to cholera toxin. LT causes diarrheae by stimulating the activity of a membrane-bound adenylate cyclase, ATP is converted into cAMP. cAMP induces the active secretion of Cl^- and inhibit the absorption of NaCl, creating an electrolyte imbalance across the intestinal mucosa, resulting in the loss of large amounts of fluid and electrolytes from the intestine.

2. Enteropathogenic *E.coli* (EPEC): Produces diarrhea by several poorly understood mechanisms. One mechanism is the production of adhesion factor which causes the adherence to the cells of the small intestine.

3. Enterohaemorrhagic *E.coli* (EHEC): Produces toxin similar to *Shigella dysenteria* (Shiga-like toxin SLT-1 and SLT-2). The toxin inhibits protein synthesis in the affected cells. The disease is known as hemolytic colitis.

Examples of serological designations *of E. coli*

- ***E. coli* O111a: H2,**
- ***E. coli* O111b: H2**
- ***E. coli* O6:**
- ***E. coli* O157: H7**

PATHOGENICITY:
Cystitis, meningitis; non-pathogenic when found in the alimentary tract of man. Epidemic diarrhea of the newborn.

NOTE: It is now widely accepted that certain serological types of E. coli are responsible for outbreaks of infantile diarrhea in nurseries.

1. Antigenic structure: O-antigen, H-antigen and K antigen

K antigen: Capsule antigen that enables the organism to resist killing by both human neutrophils and normal serum In-Vitro assays. Because K antigens is frequently found in isolates from patients with bacteriemia and neonatal meningitis, some authors feel that it plays an important role in the dissemination of organisms from primary infection sites.
2. Fimbria: Used for attachment
3. Enterotoxin (See Enterotoxigenic *E. coli*)

4. Verotoxin: Termed as such because of their irreversible cytotoxic effect on Vero tissue culture cells, a cell line developed from African green monkey kidney cells. Verotoxin producing *E. coli* (VTEC) is associated with diarrhea, hemorrhagic colitis and hemolytic-uremic-syndrome (HUS).

DIAGNOSIS:

- Stained smears reveal gram negative rods
- Triple Sugar Iron Agar A/AG
- IMVIC reaction + + - -
- On EMB : Green metallic sheen
- Lysine +
- Acetate +
- Lactose +

SEROLOGY:

Since not all strains are enteropathogenic, agglutination with specific antisera must be performed before issuing a report of isolating EPEC.

TREATMENT:
Antibiotic sensitivity testing must be performed. Aminoglycosides are effective most especially Amikacin.
Treating *E. coli* infections with antibiotics may actually place the patient in severe shock which could possibly lead to death. This is because more of the bacterium's toxin is released when the cell dies.

ENTEROBACTER

Although this bacterium is part of the normal flora of the human intestinal tract, several species cause opportunistic infections of the urinary tract as well as other parts of the body. *E. aerogenes* and *E. cloacae* are two such pathogens that do not cause diarrhea, but that are sometimes associated with urinary tract and respiratory tract infections.

SPECIES:

1. *Enterobnacter aerogenes* (Type species)
2. *E. cloacae*
3. *E. liquefaciens*
4. *E. sakazakii* (cause of diarrhea; associated with infant formula)

GENERAL PROPERTIES:

- Formerly known as aerobacter
- Often confused with Klebsiella
- Motile and ornithine decaroboxylase (ODC) positive . These tests are used to differentiate it from the similar genus of Klebsiella.

IDENTIFICATION:

A. Morphology: Short and plump rods occurring singly in pairs or in short chains; non-spore forming, motile with a peritrichous flagella.

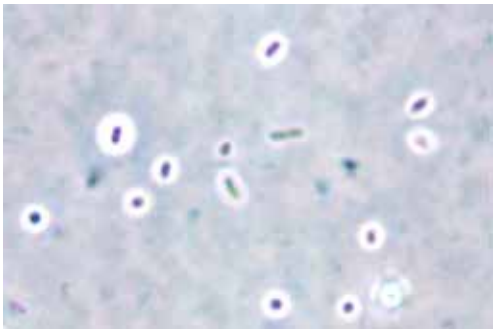
B. Culture Characteristics: Culture medium: EMB, ENDO, Mac.: On EMB, *E. aerogenes* appear as pink, while on Mac., colonies appear as pink usually not surrounded by lines of precipitated bile.

C. Biochemical characteristics:

- The organism is facultative anaerobe, reduces nitrates to nitrite
- Does not produces indole from tryptophan
- Produce acetyle methyl carbinol (Voges-Proskauer) and no H_2S
- Utilize Citrate as the source of carbon
- IMVIC reaction - - + +
- Produces A/AG (Yellow slant over yellow butt with gas).
- Positive Motility on a semi-solid medium.
- Lysine + (except *E. cloacae*)
- Ornithine +

KLEBSIELLA

The most clinically important speciies of this genus is *Klebsiella pneumoniae*. *K. pneumoniae* infections are common in hospitals where they cause pneumonia (characterized by emission of bloody sputum) and urinary tract infections in catheterized patients. In fact, *K. pneumoniae* is second only to *E. coli* as a urinary tract pathogen. *Klebsiella* infections are encountered far more often now than in the past. This is probably due to the bacterium's antibiotic resistance properties. *Klebsiella* species may contain **resistance plasmids(R-plasmids)** which confer resistance to such antibiotics as ampicillin and carbenicillin. To make matters worse, the R-plasmids can be transferred to other enteric bacteria not necessarily of the same species



SPECIES:

1. *Klebsiella pneumonia* (Type species)
2. *Klebsiella oxytoca*
3. *Klebsiella ornithinolytica*

Morphology and Staining:

- Gram-negative Bacillus
- Encapsulated (the capsule is greater in size tha
- Non-motile
- Non-spore former.

Cultural Characteristics:

Medium used for cultivation: EMB, MacConkey Agar.

= The organism develops large mucoid colonies, semifluid, with slimy appearance. Colonies exhibit positive string test.

Biochemical Characteristics:

- Urease producer (Slower and less intense than Proteus strains)
- Indole is usually (-) for *K. pneumonia*.
- Citrate is usually positive.

- TSIA A/AG
- IMVIC reaction - - + +
- Does not liquefies Gelatin.
- Lysine +
- Non-motile
- Ornithine -

Antigenic Structure:

= Klebsiella species possess O and K antigens
= The capsule enables the organism to resist phagocytosis.
= Endotoxin and enterotoxin are also produced.

Pathogenicity:

Primary community-acquired pneumonia, nosocomial pneumonia, urinary tract and wound infection, bacteremia and meningitis.

Differentiation From *Enterobacter aerogenes*

Reaction	***K. pneumonia***	***E. aerogenes***
IMVIC	- - + +	- - + +
Motility	Non-motile	Motile
String Test	Positive	Negative
Gelatine liquefaction	Positive	Negative
Ornithine decaroboxylase	Negative	Positive

CITROBACTER

Citrobacter is not considered to be an enteric pathogen because it is normal gut flora. When plated, *Citrobacter* colonies bare a strong resemblance to *E. coli* colonies. This group of bacteria is of small clinical interest. *C. freundii* is suspected to cause diarrhea and possibly extra-intestinal infections. *C. diversus* has been linked to a few cases of meningitis in newborns

SPECIES:

1. *Citrobacter freundii*
2. *Citrobacter diversus*

Characteristics:

- Morphologically appear similar to *Escherichia coli*.
- Biochemically resembles Salmonella.
- TSI: A/A+
- IMVIC - + - +
- Urease ±
- Lysine –
- Hydrogen sulfide + (*C. freundii*)
- Citrate +

SERRATIA

Members of the *Serratia* genus were once known as harmless organisms that produced a characteristic red pigment. Today, *Serratia marcescens* is considered a harmful human pathogen which has been known to cause urinary tract infections, wound infections, pneumonia and diarrhea. *Serratia* bacteria also have many antibiotic resistance properties which may become important if the incidence of *Serratia* infections dramatically increases. *Serratia* can be distinguished from other genera belonging to Enterobacteriaceae by its production of three special enzymes: DNase, lipase, and gelatinase.

SPECIES:

1. *S. marcescens*
2. *S. rubiddea*
3. *S. liquefaciens*

Biochemical Characteristics:

- Non lactose fermenter (may show delayed lactose fermentation)
- Citrate: positive.
- V-P: positive
- ODC: positive
- Lysine: positive
- Indole: Negative
- TSI A/A: (NO gas)
- DNase: positive

Specimen: Sputum, urine, and stool

Culture: A red pigment is produced by colonies of Serratia on routine laboratory media which is more clear when the organism is grown at 22 ^{o}C in the dark.

= Some strains are hemolytic.

PROTEUS GROUP

SPECIES:

1. *Proteus mirabilis*
2. *Proteus penneri*
3. *Proteus vulgaris*
4. *Morganella morganii*
5. *Providencia alcalifaciens*
6. *Providencia stuartii*
7. *Providencia rettgeri*

MORPHOLOGY:

- Gram negative Bacilli
- Actively motile
- No capsule
- No spores

CULTIVATION:

On EMB, Endo and MacConkey Agar

The colonies usually exhibit swarming. Non-lactose fermenting (Colorless).

Pathogenicity:

Proteus, like almost every other bacterium in this family, can cause urinary tract infections and hospital-acquired infections. *Proteus* is unique, however, because it is highly motile and does not form regular colonies. Instead, *Proteus* forms what are known as ***"swarming colonies"*** when plated on non-inhibitory media. The most important member of this genus is considered to be *P. mirabilis*, a cause of wound and urinary tract infections. Fortunately, most strains of *P. mirabilis* are sensitive to ampicillin and cephalosporin. Unlike its relative, *P. vulgaris* is not sensitive to these antibiotics. However, this organism is isolated less often in the laboratory and usually only targets immunosuppressed individuals. *P. mirabilis* and *P. vulgaris* can be differentiated by an indole test for which only *P. vulgaris* tests positive.

Diagnosis:

- Smear: Gram-negative bacilli
- TSIA K/AG+
- IMVIC V + - -
- Swarming and "Gun-powder-like odor"
- Strong urease positive.
- Phenylalanine Deaminase test = Positive
- Lysine –
- Hydrogen sulfide +
- Motile

NOTE: Proteus strains are used in the Weil-Felix test for Rickettsial diseases:

1. Proteus OX_K
2. Proteus OX_{19}
3. Proteus OX_2

Weil and Felix isolated strains of *Proteus vulgaris* from patients with typhus fever and found that the sera of the patients agglutinated particular Proteus strains designated OX_{19} and OX_2. The Proteus organism is not the cause of Typhus fever. The cross agglutination seems to be caused by the presence of an alkali-stable polysaccharide which is also present in *R. prowazeki*. The agglutination of these particular strains by sera of patients with Rickettsial infection is known as the "Weil-Felix reaction" .

Therefore Proteus does not cause Typhus fever nor acts as a secondary invaders .

Morganella and Providencia

Both are closely related to the Proteus group because both elaborate **urease** and produce **indole**. They however, **don`t produce hydrogen sulfide**.

Morganella morganii

Morganella morganii is the only important species of this genus. It can cause urinary tract and wound infections, as well as diarrhea. Chloramphenicol is a good choice for treating *Morganella* infections.

- Ornithine +
- TSIA: A/A
- IMVIC reaction : + + - -

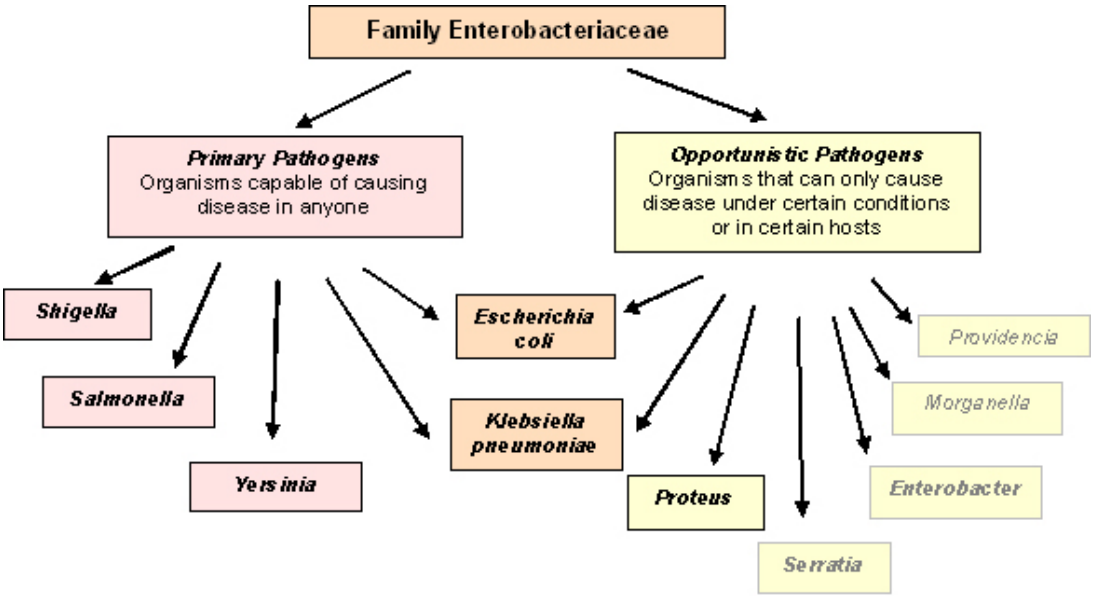
Providencia alcalifaciens

Although rare, *Providencia* species have been associated with nosocomial (hospital acquired) urinary tract infections. One species, *P. alcalifaciens*, has been associated with some cases of diarrhea in children. Since infection is so rare, other genera of Enterobacteriaceae should be considered before *Providencia* as being causative.

- Hydrogen sulfide -
- Lysine -
- Lactose -
- TSIA: K/A
- IMVIC reaction: + + - +

ERWINIA

Not medically important since they are only pathogenic to plants.



SALMONELLA

SPECIES OF MEDICAL IMPORTANCE:

1. *S. typhi*	(The typhoid bacilli)
2. *S. paratyphi A*	(Produce paratyphoid fever)
3. *S. paratyphi B*	(Produce paratyphoid fever)
4. *S. paratyphi C*	(Bacteremia without intestinal involvement)
5. *S. typhimurium*	(Food poisoning)
6. *S. enteritidis*	(Food infection)
7. *Salmonella cholerasuis*	(Hog cholera bacillus)
8. *Salmonella pullorum*	(White diarrhea in children)
9. *Salmonella gallinarum*	(Fowl typhoid bacillus)

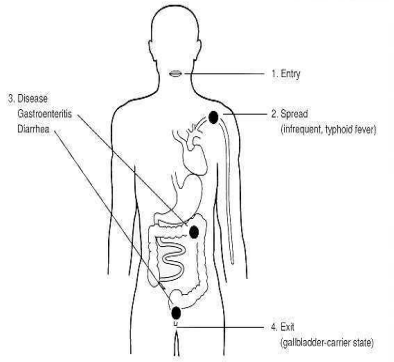
MORPHOLOGY AND STAINING:

- Short rods (bacilli) (indistinguishable from other Enterobacteriacae)
- Motile with peritrichous flagella except *S. pullorum* and *S. gallinarum.*
- Non-encapsulated
- Gram-negative

HABITAT AND TRANSMISSION:

Most Salmonella species are found in the intestine of animals especially pigs, cows, goats, sheep, rodents, hens, ducks and other poultry. *S. typhi* and *S. paratyphi*, however ,are usually found only in human., both of which are excreted in the feces and urine of infected patients. Infection occurs via ingesting contaminated food or drinks.

PATHOGENICITY:



Salmonella can cause any one of three types of Salmonellosis:

1. Acute gastroenteritis or food infection type.
2. Septicemia or acute sepsis similar to pyogenic infection.
3. Enteric fevers:
    1. Typhoid fever
    2. Paratyphoid fever.

Typhoid fever is an acute infectious disease characterized by continuous fever, skin eruptions, bowel disturbances and profound toxemia. Following entrance of the typhoid bacilli into the human body through the mouth there is always an incubation period of 7 to 14 days, before symptoms appear. During this time, the organism penetrates the wall of the upper intestine and causes and inflammation. Then reaches the blood via the lymphatic system were they circulate and may be localized in many internal organs especially in spleen, bone marrow, and gallbladder. Serious complication of typhoid fever may be produced as a result of multiplication of the bacilli in organs other than the intestine.

The organism begin to disappear from the blood during the first week of the illness, and especially after the second week. The disappearance of the organism from the blood is clearly associated with the development of specific antibodies.

NEPHROTYPHOID: This condition is an immune complex disorder of the kidneys and is characterized by fever, edema, marked albuminuria and hematuria.

OSTEOMYELITIS : Especially in children with sickle cell disease and thalassamia. Typhoid in children can be found in the bone marrow. Inflammation of the joints (Typhoid arthritis) may also occur.

ABSCESSES: of the spleen and elsewhere.

MENINGITIS: And rarely pneumonia and endocarditis.

DETERMINANTS OF PATHOGENICITY:

1. Surface antigens: The ability of Salmonella to attach to host receptor cells and to survive intracellularly may be due to the O antigenic side chain or in case of typhi serotypes, the presence of Vi antigen. Organisms containing Vi antigen are clearly more virulent than those lacking this antigen. It may function as a capsule and prevent phagocytosis or intracellular destruction of the organism.

2. Invasiveness: Virulent Salmonella penetrate the epithelial lining of the small bowel, thus the brush border begins to degenerate. After penetration the organism multiply and may spread to other body sites.

3. Endotoxins: Presumably, endotoxin is responsible for the fever production. Endotoxin activation of the chemotactic properties of the complement system may cause the localization of leukocytes in the classic lesions seen in typhoid fever.

4. Other factors:
Enterotoxin: Affects the small intestine.
Cytotoxin: Associated with outer bacterial membrane which may mean that the toxin may be important in cellular invasion and cellular destruction.

CLASSIFICATION OF SALMONELLA:

The Kauffman-White system used to classify Salmonella is based on identifying the O (somatic) and H (flagellar) antigens possessed by the different serovars. The detection of Vi antigen is also used in the identification of *S. typhi* and some other Salmonella.

1. O Antigen: Salmonella are grouped by their O antigens as groups A to Z, 51-61 and 64-66. Many of the medically important salmonella belongs to groups A to G. Each group has what is called a group factor. This is an O antigen, common to all members of the groups. For example, the factor for group B is O antigen is 4. This means that all the salmonella belonging to this group possess antigen 4 as one of their O antigen.

2. H Antigen: Many Salmonella are diphasic, that is, they can occur in two antigenic forms referred as phase I and Phase II. Phase I antigens are given alphabetical letters and phase II antigens either numbered or given a letter if known to occur in both phases. Phase I antigens are more specific and therefore, an organism can be identified if it is in phase I.

3. Vi Antigen: This surface (K) antigen can be found in *S. typhi* and *S. paratyphi C* and few other Salmonella. It is associated with virulence and can be detected using Vi antiserum.

SALMONELLA	O	H I	H II
Group A: *S. paratyphi A*	1, 2*,12	a	-
Group B: *S. paratyphi B*	1,4*,5,12	b	1,2
S. derby	1,4*,5,12	f,g	1,2
S. typhimrium	1,4*,5,12	i	1,2
S. heidlberg	1,4*,5,(12)	r	1,2
Group C: *S. cholerae-Suis*	*6, 7	c	1,5
S. paratyphi C	6*, 7 (Vi)	c	1,5

LABORATORY DIAGNOSIS:

1. SPECIMEN: For the diagnosis of enteric fever, specimens include, blood, feces and urine for culture may be used depending on the course of illness.
A. Blood: Organisms can usually be detected in 75-90% of patients during the first 10 days of infection and in about 30% of patients during the 3rd. week.
B. Feces: Organisms can usually be isolated from 40-50% of patients during the third week.
C. Urine: Organism, can usually be detected from about 25% of patients after the second week of infection.
D. Serum: Is used for the detection of serum antibodies (Widal test).

GROUPING AND SEROTYPING OF SALMONELLA

Due to the high cost of antisera, it will not be possible for most laboratories to stock wide range of Salmonella antisera, however, laboratories try to stock a salmonella polyvalent O antiserum which covers the locally important group and also specific O, H, and Vi antisera to identify *S. typhi.*

The following antisera are required to identify S. typhi
1. Salmonella antiserum Factor 9 (Group D)
2. Salmonella H antiserum d
3. Salmonella Vi antiserum.

If testing for paratyphoid the following antigen suspensions are required:
1. *S. paratyphi* A O1, 2, 12
2. *S. paratyphi* A H,2
3. *S. paratyphi B* O1,4,5,12
4. *S. paratyphi* H,b, phase I
5. *S. paratyphi* C O6,7
6. *S. paratyphi* C H, c, phase I

2. ISOLATION AND IDENTIFICATION:

A. ENRICHMENT & SELECTIVE MEDIA:

Various enrichment and selective media are used to isolate Salmonella from stool and other specimens. The use of Selinite-F and XLD, DCA and SSA. Salmonella produce non-lactose fermenting colonies on MacConkey medium. Most strains (especially food-poisoning serovars) show a blackening of the colonies due to H_2S production.

= Enrichment medium:

Contains substances which have a growth stimulating of Salmonella and Shigella and inhibitory to the other contaminants. Ex. Selenite-F Broth
Tetrathionate broth.

= Selective media:

Solid media containing lactose as differential sugar; an indicator to produce color changes when the pH of the colony becomes acid as a result of lactose fermentation and an inhibitor for gram positive bacteria and most gram-negative bacteria other than Salmonella and Shigella.

1. Bismuth Sulfide Agar (BSA): Considered by many as the best medium for the isolation of *Salmonella typhi*.
2. Brilliant Green Agar (BGA): This medium is good for isolating Salmonella species other than *S. typhi*.
3. Salmonella Shigella Agar (SSA): Colorless colonies .
4. Desoxycholate Citrate Agar (DCA).
5. Xylose Lysine DextroseAgar (XLD).

B. GROWTH CHARACTERISTICS

1. On MacConkey and SSA : Colorless colonies
2. BGA : Slightly pink colonies
3. BSA and XLD : Develops black colonies

C. BIOCHEMICAL CHARACTERISTICS

A. TSIA: Alkaline slant, Acid butt with small amount of H_2S
B. IMVIC reaction: - + - +
C. Motility (+)
D. Urease (-)

D. SEROLOGICAL TESTING:

1. On clinical isolates: All clinical suspected Salmonella isolate must be subjected to serotyping by polyvalent and monovalent antisera.
2. On patients: Serological test (Widal test) must be performed after 10 days of infection .

TREATMENT:

ENTEROCOLITIS

- Rehydration
- Antimicrobials not indicated

ENTERIC FEVER

- Chloramphenicol was firstly used in 1948 for typhoid fever - Now resistant
- Ampicillin, sulphamethoxazole-trimethoprim still used in developing countries.
- Ciprofloxacin – drug of choice
- Rehydration if needed

CHRONIC CARRIER

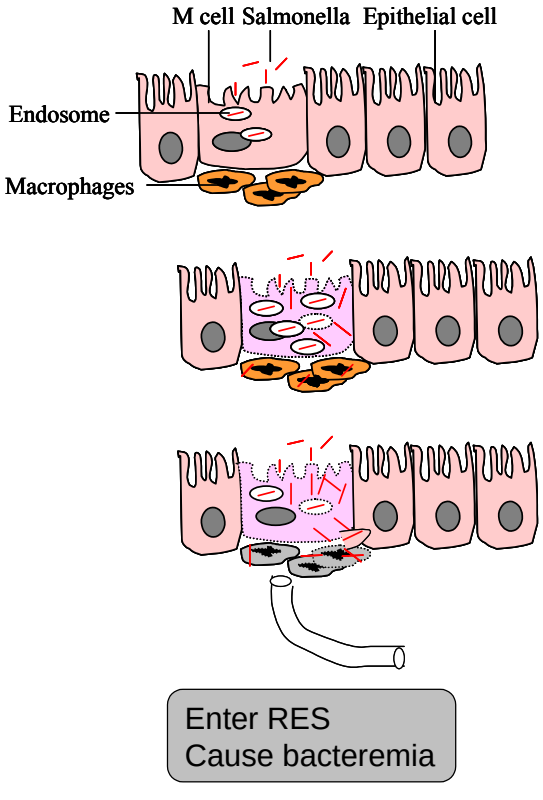
- Prolonged treatment with Ampicillin or Ciprofloxacin
- Cholecystectomy may be needed

Prevention

- Public health = personal hygiene measures
- Proper sewage treatment
- Chlorination of water supplies
- Detection of carriers
- Pasteurization of milk and proper cooking of food

Vaccination (50-80%) protection) – two vaccines:

- Acetone-killed – given I/M
- Live-attenuated - Oral



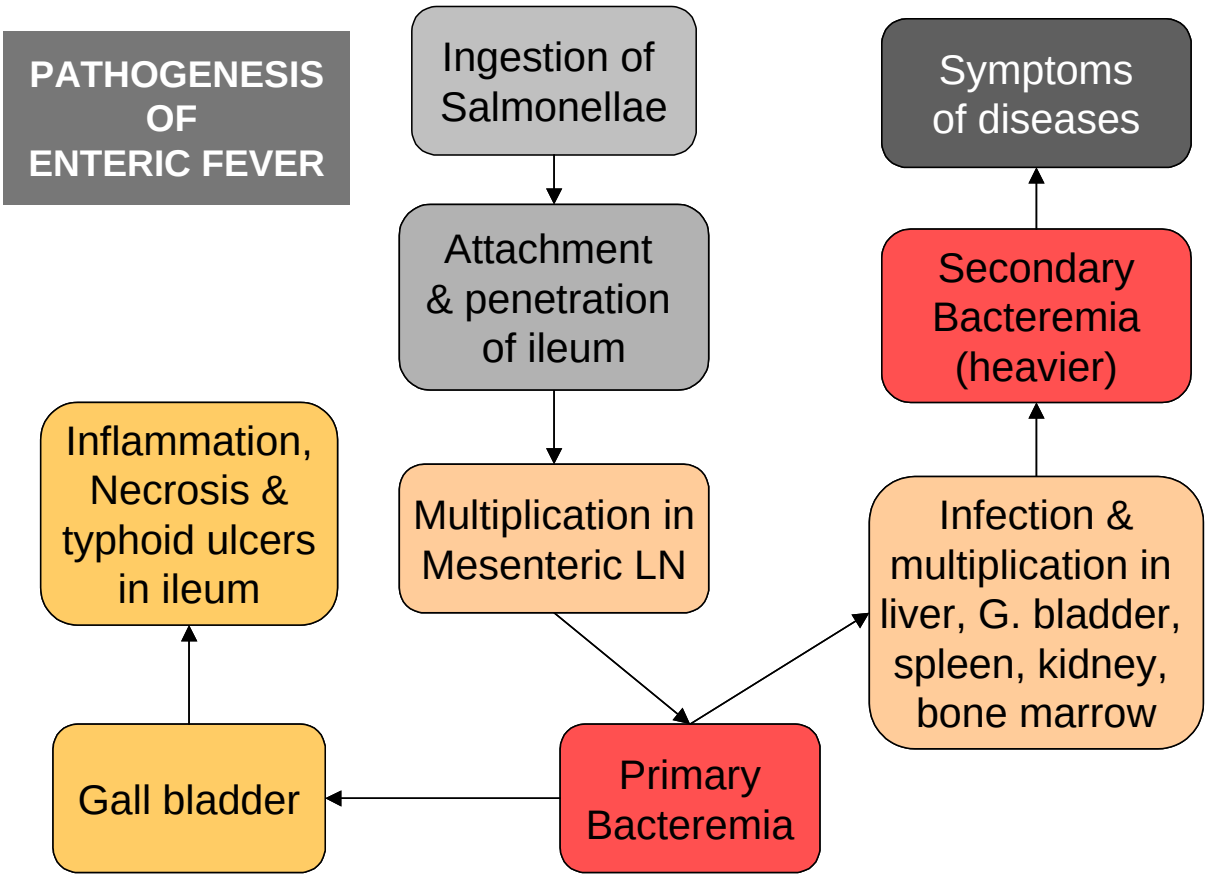
Pathogenesis of Enteric Fever

Salmonella are taken up by M cells in the small intestine by endocytosis.

Bacteria multiply within endosomes and kill M cells. Bacteria are discharged from base of cell by exocytosis and are taken up by macrophages in Peyer's patches.

The bacteria multiply inside cells leading to death of macrophages. Are carried via lymphatics to macrophages in mesenteric LN, liver, spleen, bone marrow where they multiply & cause bacteremia.

Symptoms of the disease are probably due to endotoxins



SHIGELLAE

- The genus Shigella contains fewer species than the genus Salmonella and is antigenically less complex.
- Clinical dysentery may be caused by *Shigella*, *Salmonella*, *Entamoeba histolytica*, *Proteus morganii*, and viruses.
- Shigella dysenteria was isolated by Shiga in Japan in 1898.

SPECIES:

1. *Shigella dysenteriae*
2. *Shigella flexneri*
3. *Shigella sonnei*
4. *Shigella boydii*

CLASSIFICATION:

1. Non-mannitol-fermenters
   Shigella dysenteria
2. Mannitol-fermenters
   Shigella flexneri
   Shigella boydii
   Shigella sonnei

MORPHOLOGY AND STAINING:

- Short rods
- Non-encapsulated
- Non-motile
- Non-spore former
- Gram-negative

HABITAT AND TRANSMISSION

Shigella species are found only in the human intestinal tract.
Carriers of pathogenic strains can excrete the organism up to two weeks after infection and occasionally for longer periods.
Shigella are killed by drying.
Shigella are transmitted by the fecal-oral rout.
The highest incidence of Shigellosis occur in areas of poor sanitation and where water supplies are polluted.

CULTURAL CHARACTERISTICS:

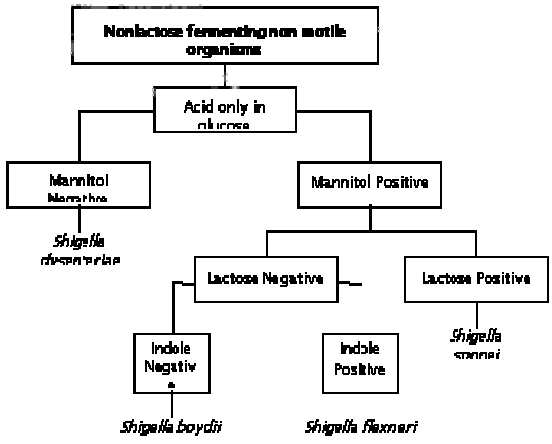
All members of Shigella are aerobic and facultative anaerobes. Grow readily in culture media at pH 6.4 to 7.8 at 10 ^{o}C - 40 ^{o}C, with optimum of 37 ^{o}C.
After 24 hours incubation, Shigella colonies reaches a diameter of about 2 mm.
The colonies are circular, convex, colorless, but moderately translucent with smooth surface, and entire edges.

Small tangled hair-like projections can sometimes be seen at one or more points on the periphery of the colony. I
n XLD they appear pinkish to reddish colonies while in Heaktoen Enteric Agar (HEA), they give green to blue green colonies.

If a number of typical colonies present onto the original plate, a tentative diagnosis can be made by direct slide agglutination with polyvalent Shigella antiserum.
In all instances, diagnosis should be confirmed by additional biochemical tests and by specific type agglutination.

BIOCHEMICAL CHARACTERISTICS:

=All ferment glucose, some ferments mannitol
=They do not form acetyl-methylcarbinol,
=Does not hydrolyze urea or liquefy gelatin
=Citrate negative
=TSIA (Alkaline slant over acid butt)



=IMVIC V + - -

PATHOGENIC DETERMINANTS:

1. O antigen: The ability to survive the passage through the host defenses may be due to O antigen.

2. Invasiveness: Virulent shigella penetrate the mucosa and epithelial cells of the colon in an uneven manner.

Intracellular multiplication leads to invasion of adjacent cells, inflammation and cell death.

Cell death is probably due to cytotoxic properties of shiga toxin that interfere with protein synthesis.

The cellular death and resulting phagocytosis response by the host accounts for the bloody discharge of mucus and pus and shallow ulcers characteristic of the disease.

3. Other toxins: It has a protein toxin which may be neurotoxic, cytotoxic, and enterotoxic. The enterotoxic property is responsible for watery diarrhea.

PATHOGENICITY:

Shigella dysentery's is set apart from other dysentery bacilli by its capacity to form a powerful exotoxin, it is associated with epidemics of bacillary dysentery. It is the only dysentery bacillus that is pathogenic to laboratory animals.

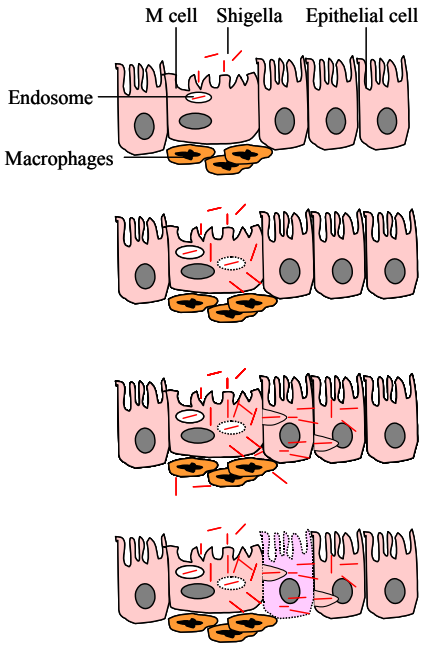
In man, shigellosis begins with symptoms of acute gastro-enteritis which is accompanied by abdominal pain and diarrhea.

As it progresses, diarrhea becomes more frequent and is usually accompanied colicky pain.

Later diarrhea losses its fecal characteristic and is followed by mucus with pus and blood.

The disease is usually accompanied by fever and marked prostration.

It is also known that children are more frequently attacked than adult persons and the symptoms are more severe.



Pathogenesis of Shigella

Shigella are taken up by M cells in the large intestine by endocytosis.

Bacteria are quickly released from endosomes and leaving shigella free in the cytoplasm. The bacteria multiply & enter the inferior and lateral aspects of the epithelial cells

Actin filaments quickly form a tail and push the bacteria into next cell where they multiply. Macrophages that take up shigella are killed and release organism.

Infected cells die and slough off. Acute inflammatory respose occurs with with bleeding and abscess formation.

LABORATORY DIAGNOSIS:

The only satisfactory method of laboratory diagnosis is to cultivate the bacilli from the patient.

In the early stages of acute shigellosis, isolation of the causative organism from the feces is usually accomplished without difficulties by using the same special media and methods employed for salmonella.

1. Cultivation of the bacilli from stool specimen during the first 4-5 days of the disease.
2. Smears: Gram-negative bacilli appearing singly

TSIA = Alkaline/acid (No gas no H_2S)

IMVIC reaction : V + - -

3. Serological examination with polyvalent and monovalent anti-sera.

METHODS FOR THE IDENTIFICATION OF SALMONELLA AND SHIGELLA

1. Shigella are rarely encountered except in feces, Salmonella are also found in cultures of bile, blood, urine, abscesses and cerebrospinal fluid.
2. Microscopic examination of stained smears is of no use for the identification of the bacilli except the fact that it is a gram negative bacilli.
3. On general and selective media colonies are similar. On Blood agar they are smooth, gray and opaque. Most salmonella species produces H_2S and therefore will be black on Bismuth Sulfite Agar.

POINTS OF IDENTIFICATIONS:

FEATURE	SALMONELLA	SHIGELLA
Disease	Typhoid fever	Bacillary dysentery
Motility	Motile	Non-motile
H2S	Positive	Negative
Indole formation	Positive	Negative

TSIA reaction	K/A+	K/A
Nature of infection	Systemic	GIT
Immunity	Lasting	Short period
Metabolite	Potent Endotoxin	endo & exotoxin

***NOTE*: For rapid differentiation of Shigella from other Non-lactose fermenting bacilli by the following test (Motility-Indole-Urea) All are negative.**

= Difco (Bacto) Shigella antisera:
Polyvalent group A = Reacting with *S. dysenteria* 1-7
Polyvalent group A1 = Reacting with *S.dysenteria* , 8ab, 8ac, 9,10
Polyvalent group B = Reacting with *S. flexneri*, 1-6
C1 = Reacting with *S.boydii* 1-7
C2 = Reacting with *S.boydii*,8-11
C3 = Reacting with *S.boydii*,12-15
Polyvalent group D = Reacting with *S. sonnei*

TREATMENT:
1. Water and electrolytes replacement
2. Antibiotic therapy is required to eliminate the organism. Due to the emergence of resistant strains of shigella, antibiotic sensitivity, must be performed on any shigella isolate to determine suitable antibiotics:

Sulfonamides, tetracycline, Chloramphenicol, ampicillin and streptomycin are known to be effective against shigella.

Immunity:

- Short lived; Preparation of oral live attenuated vaccine is on the way to stimulate mucosal IgA.

Prevention

- Sanitary precautions
- Good personal hygiene (hand washing)

YERSINIA

SPECIES OF MEDICAL IMPORTANCE:

1. *Yersinia pestis* Plague
2. *Yersinia enterocolitica* Yersinosis
3. *Yersinia pseudotuberculosis* Yersiniosis

General Characteristics:

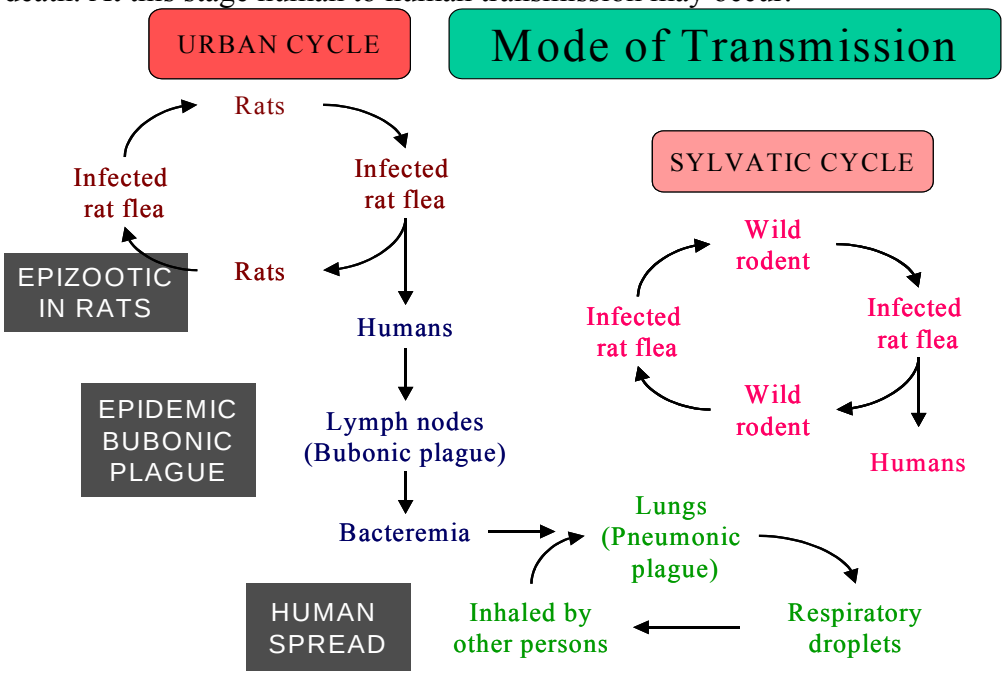
- Gram-negative rods (Coccobacilli)
- Non-motile with the exception of *Y.pseudotuberculosis* and *Y. enterocolitica* (motile at 22^{o}C)
- Facultative anaerobe
- Catalase positive
- Oxidase negative

Yersinia pestis

Clinical manifestation:

Plague is primarily a disease or rodents that could be transmitted to man. Plague occurs in three forms: **BUBONIC, SEPTICEMIC and PNEUMONIC.** Bubonic plague is the common form resulting from the cutaneous inoculation of *Y. pestis* by the bite of an infective flea. The incubation period is 2-7 days after exposure. The disease is characterized by sudden fever, shaking chills, headache and pain in the area of involved lymph nodes. Untreated, bubonic plague can result in coma and death. Classic bubonic plague entails an inflammatory response in the regional lymph nodes which, when enlarged, is referred to as bubo.

Untreated bubonic plague may become septicemic plague. Virtually, 100% of the untreated septicemic plague infections are followed by pulmonary involvement and death. At this stage human to human transmission may occur.



NB: Plague is an internationally reportable disease, and when suspected, must be reported to the local public health authorities.

Collection of Specimen:

An aspirate from buboes is a good specimen if handled properly using a sterile syringe and needle and aseptic technique in collecting. Special precautions must be taken to avoid the spread of infection. Face masks, gloves and gowns must be routinly worn when in contact with the patient.

Blood drawn aseptically is also good specimen for isolation of *Y.pestis* is septicemic patients.

Early morning sputum obtained from deep couph, avoiding contamination with saliva. The patient must be instructed to rinse the oral cavity three to four times.

Biopsy from infected lymph nodes.

Staining & Culture Media:

= Smears are stained with **WAYSON** to be examined for bipolar stained rods.

= Routine media, selective for gram negative enteric bacteria are good for isolation. **BHIB, Trypticase Soy Broth, Blood Agar** and **Nutrient Agar** are also used.

Identification by Biochemical Tests:

= *Yersinia pestis* is differentiated from other Yersinia species by a negative urease negative test and carbohydrate fermentaion tests. Motility at 37°C and 22°C is also used.

Serologic identification:

1. Identification of suspected *Y. pestis* isolate: Commercially avialable antiplague serum is used to agglutinate cells.
2. Patient serum: (Detection of antibodies): Formalinized suspension of known *Y. pestis* cells is used as antigen. One drop of diluted antigen is added to one drop of patient`s serum. This mixture is shaken gently for 15-30 minutes and examined for agglutination under low power magnification.

Antimicrobial Sensitivity testing:

Routine sensitivity testing is unreliable because In-Vitro results commonly differ significantly from In-Vivo efficacy. For example, penicillin may show slight to wide zone of inhibition of *Y. pestis*, although penicillin has no In-Vivo activity against this organism. Tetracycline, Streptomycin or Chloramphenicol and sulfonamides are effective in treatment of plague.

II *Y. enterocolitica* and *Y. pseudotuberculosis*

Clinical manifestation:

Both agents causes yersinosis which appears as enteric disorders, e.g., diarrhea, enteritis, terminal ileitis. Clinical symptoms can not be distinguished from those caused by other enteric pathogens such as salmonella and shigella. Faecal-oral rout is the common mode of transmission, although may be transmitted by bites of infective arthropods. Human to human transmission is well documented.

Collection & processing of Specimen:

= Stool, blood, excised mesenteric nodes, or appendices obtained during surgery.

= Yersinia Selective Agar: is an excellent selective medium for the isolation of *Y. enterocolitica* from stool or sputum.

= Cold Enrichment technique: Specimens that are grossly contaminated, are inoculated into buffered saline (pH 7.4-7.6) and stored at 4-7 °C. Both organisms tolerate and even grow at these temperatures, other organisms gradually dies.

Biochemical Identification:

1. *Y. enterocolitica*

= Growth at 4 °C and on NA/Mac. Agars
= Motile at 22 °C.
= Indole production= Variable
= Urease positive
= Ornithine decarboxylase Positive

2. *Y. pseudotuberculosis*

= The same as *Y.enterocolitica* and could be differentiated from it by a sorbitol negative test and negative ornithine decarboxylase.

Serology:

= Specific antigens prepared from each organism is useful in detecting the presence of specific antibodies in patient`s serum.
= Specific antibodies are used to agglutinate suspected cultures.

Treatment:
Streptomycin, Chloramphenicol, Tetracyclines.

Gram Negative Bacterial Reactions	Triple Sugar Iron Agar				Urease	Citrate	SIMS		MR	VP	Nitrate	Lysine Iron Agar	
	Slant	Butt	Gas	H_2S			Indole	H_2S				Slant Deamination	Butt Decarb-oxylation
Escherichia coli	A	A	+	-	-	-	+		O+	-	+	-	+
Enterobacter aerogenes	A	A	+	-	-	+	-		-	+	+	-	+
Aeromonas hydrophila	A	A	+	-	-	+	+		-	-	+	-	+
Klebsiella oxytoca	A	A	+	-	+S	+	+		-	+	+	-	+
Klebsiella pneumoniae	A	A	-	-	-	+	-		O+	-	+	-	+
Citrobacter freundii	A	A	+	+	-	+	-		O+	-	+	-	-
Proteus mirabilis	Alk	A	+	+	+	+	-		-	-	+	+	-
Proteus vulgaris	A	A	+	+	+	-	+		-	-	+	+	-
Morganella morganii	Alk	A	+	-	+	-	+		-	-	+	+	-
Pseudomonas aeruginosa	Alk	Alk	-	-	-	+	-		-	-	+Z	-	+
Psueduomonas putida	Alk	Alk	-	-	-	+	-		-	-	-Z	-	+
Bergeyella zoophlecum	Alk	Alk	-	-	+	+	-		-	-	+	-	+
Acinetobacter lwoffii	Alk	Alk	-	-	-	-	-		-	-	-Z	-	+
Providencia rettgeri	Alk	A	-	-	+	+	+		O+	-	+	+	-
Providencia alcalifaciens	Alk	A	-	-	-	+	-		-	-	+	+	-
Shigella boydii	Alk	A	-	-	-	-	-		+	-	+	-	-
Salmonella cholerasuis	Alk	A	+	+	-	+	-		+	-	+	-	+
Salmonella typhimurium	Alk	A	+	+	-	+	-		O+	-	+	-	+

Key S = slow O = orange/red Z = zinc

PSEUDOMONAS AND MISCELLANEOUS NON-FERMENTERS

The genus Pseudomonas is rather a heterogeneous group of microorganisms, with few phenotypic characteristics to clearly distinguish it from other genera.

I. Pseudomonas:

Pathogenic species:

1. *Pseudomonas aeruginosa*
2. *Ps. pseudomallei*
3. *Ps. mallei*

1. *Pseudomonas aeruginosa*

May grow in:

- Disinfectants
- Contact lens solutions
- Medicinal solutions
- Humidifiers of respirators

GENERAL CHARACTERISTICS:

1. Obligate aerobic, gram negative bacilli indistinguishable from Enterobacteriaceae.
2. Non-lactose fermenter
3. Oxidase positive
4. Glucose oxidizer
5. Catalase positive
6. Citrate positive
7. Motile by a polar flagella.
8. Produces exotoxin A = Inhibits protein synthesis and produces tissue necrosis.
9. Produces collagenase which hydrolysis collagen
10. produces elastase which hydrolysis elastin and is used by the organism for invasion.
11. It produces pigments
    - 11.1 **pyocyanin** = blue green pigment
    - 11.2 **pyoveridin** = Yellow- fluorescence.

NB: These pigments could be detected by wood's Ultraviolet Light.

PATHOGENICITY:

Ps. aeruginosa is an opportunistic pathogen that infect immunocompromized patient. Usually causes hard to treat nosocomial infections. It show resistance to most antibiotics.

DISEASES caused by *Ps. aeruginosa* include:

1. Urinary tract infection (UTI)
2. Otitis media
3. Wound infection
4. Sinus infection
5. Bronchopneumonia
6. Sepsis
7. Burn infection
8. Meningitis
9. Endocarditis

IDENTIFICATION:

1. Gram staining:

Ps. aeruginosa is indistinguishable from other gram negative bacilli. Therefore there is a little significance for gram staining.

2.Culture:

Ps. aeruginosa grow well on ordinary media such as Blood Agar, Nutrient Agar and MacConkey Agar.

= Colonies are 2-4 mm in diameter, slightly convex, grayish the smell like grape and are often hemolytic.

= On Nutrient Agar, *Ps. aeruginosa* is easily recognized by the diffused blue-green pigmentation.

Biochemical tests:

See table one for distinguishing tests and also refer to the general characteristics.

Table 1. Differential Characteristics of the Common Non-Fermenting Gram-Negative Bacilli.

GENUS	OXIDASE	GLUCOSE OX.	FLAGELLA
Pseudomonas	V	V	Polar
Achromobacter	+	+	Peritrichous
Flavobacterium	+	+	None
Kingella	+	+	None
Brucella	+	V	None
Alcaligenes	+	-	Peritrichous
Bordetella	V	-	(V) peritrichous
Moraxella	+	-	None
Acinetobacter	-	V	None

+ Positive, - Negative, V Variable

4. Serological typing: This is performed through an agglutination test based on the reactivity of somatic O antigens of intact cell with specific antisera. Cross reactivity may occur in 10-11% of cases.

5. Pyocin typing: Pyocin is an antibiotic produced by Pseudomonas aeruginosa that can inhibit the growth of other bacterial species. This test is less commonly used.

ANTIBIOTIC SUSCEPTIBILITY:

Ps. aeruginosa is typically resistant to commonly used antibiotics.

= Aminoglycosides (Amikacin, gentamicin), polymyxins and colistin are used in the treatment of this organism. 3rd & 4th generation cephalosporins

= Mg++ and Ca++ must be added to the growth medium used in the sensitivity testing.

2. *Pseudomonas pseudomallei*

DISEASE: Melioidosis: Uncommon tropical disease = Pulmonary manifestation of the disease which is usually confused with tuberculosis.

Characteristics:
1. Non pyocyanin producer
2. Esculin hydrolysis = Positive
3. Lactose oxidation = Positive

NB: These characteristics are used to differentiate it from *Ps. aeruginosa.*

3. *Pseudomonas mallei*

DISEASE: Glander: Ulcerating, tubercle-like nodules forms in the lungs, superficial lymph nodes and mucus membrane.
= This organism could be differentiated from other Psudomonas species by carbohydrate oxidation test.

II. Acinetobacter

Pathogenic species:
1. Acinetobacter calcoaceticus

DISEASE: It is an opportunistic pathogen. In patients with burns or with immunologic deficiency, they can produce sepsis or pneumonia.
Diagnostic tests: On gram stained smears it mimics the appearance of Niesseria and thus sometimes called (mima), but it is rapidly differentiated from Niesseria by negative Oxidase test.
1. Non motile
2. Resistant to penicillin
3. Grow well on MacConkey Agar.

TREATMENT:
It is resistant to penicillin G but usually respond well to gentamicin, amikacin and minocycline.

III. Achromobacter

Pathogenic species:
1. *Achromobacter xylosoxidans* now known as *Alcaligenes denitrificans*
DISEASES:
1. Septicemia 2. Otitis media
3. Meningitis 4. Wound infection
5. Urinary tract infection.

CHARACTERISTICS:
1. It grow well on MacConkey Agar
2. Oxidase positive
3. Motile by peritrichous flagella
4. Acidification of Oxidation-Fermentation (OF) glucose and xylose.

IV. Alcaligenes

Pathogenic species:
1. *A. faecalis* = recovered from blood, sputum and urine
2. *A. odorans* = recovered from urine
3. *A. denitrificans*

CHARACTERISTICS:
1. Motile by peritrichous flagella
2. Oxidase positive
3. Hydrolysis urea
4. Citrate positive
5. Inability to utilize OF carbohydrates

Table.2 Discriminating Characteristics of Members of the Genus Alcaligenes

Characteristics	*A. faecalis*	*A. odorans*	*A. denitrificans*
Nitrate reduction	+	-	+
Nitrite reduction	V	+	+
Denitrification	-	-	+
Growth in 6.5% NaCl	-	+	+
Fruity odor	-	+	-

Treatment:
Susceptible to cephalosporins, such as carbenicillin. Also to minocycline, doxycycline and polymyxin B.

GENUS: VIBRIO

SPECIES:

1. *Vibrio cholera* O1 (The main species of medical importance)
2. *Vibrio mimicus* (Very similar to *V. cholera*)
3. *Vibrio parahemolyticus*
4. *V. vulnificus*

General Characteristics:

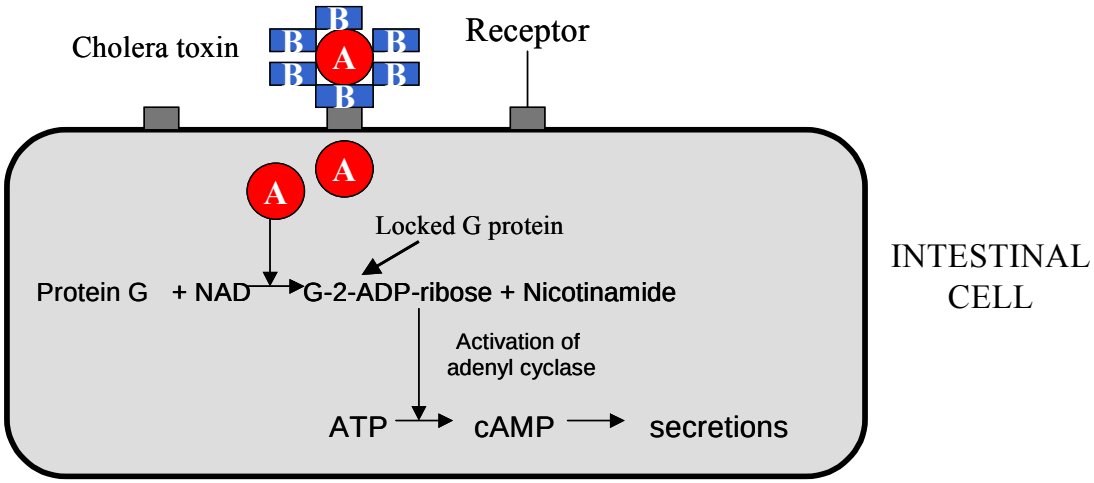
The genus Vibrio is composed of a morphologically related species characterized by:

1. Small gram negative curved rods
2. Motile by polar flagella
3. Aerobic and facultative anaerobes

PATHOGENICITY:

V. cholera adheres to the walls of the intestine without invading it. The organism produces a powerful enterotoxin (Exotoxin) that activate the enzyme adenylcyclase. This leads to the secretion of large volumes of fluids and electrolytes into the leumen of the intestine. Watery diarrhea is produced. Rice water stool containing vibrios, epithelial cells and mucous are passed. In acute choleral cases, the rapid loss of fluids and electrolytes leads to muscular cramps and severe dehydration which if not treated may be fatal.

Pathogenesis of Cholera Toxin



ADP-ribosyulation of Protein G

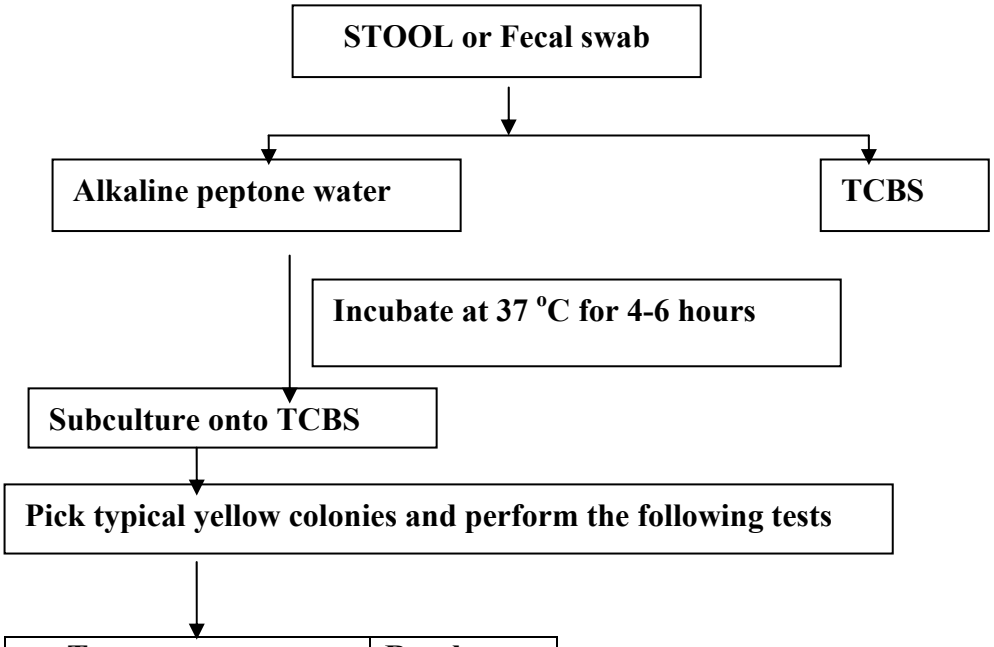
- Subunits B facilitate entry of subunit A (Active subunit) into cell
- Subunit A cleaves nicotinamide from NAD and transfers the remaining ADP-ribose to protein G (locked protein G)
- Activates adenyl cyclase to convert ATP to cAMP - secretions

THE ORGANISM:

- Best growth in alkaline conditions (pH 8-9.5)
- Alkaline peptone water (pH8.6) - rapid isolation from feces
- Selective medium : TCBS
- Oxidase positive
- Gram-negative curved rods
- Single flagellum at one end (actively motile)
- Carbole fuchsin is recommended as a counter stain in the gram staining technique when staining vibrio species.

SPECIMEN & MEDIA:

=Stool or fecal swab
= Media A: Enrichment = Alkaline peptone water (pH 8.6)
B: Selective = TCBS : Thiosulfate Citrate Bile Salt Sucrose Agar
= In alkaline peptone water: Growth occur within within 4-6 hours.
= In TCBS: Yellow colonies are produced as a results of sucrose fermentation.



Test	Result
Oxidase test	Positive
Catalase test	Positive
Esculin	Negative
Motility test	Positive
ONPG	Positive
Indole	Positive
Voges-Proskauer	+ or -
Sucrose fermentation	Positive
Lactose	Negative
Mannitol	Positive

PRESUMPTIVE IDENTIFICATION OF *VIBRIO CHOLERA:*

1. Isolation of sucrose fermenting colonies (yellow) colonies on TCBS.
2. Motility positive and gram negative rods
3. Positive oxidase test
4. Positive **string test:** A typical yellow colony is rubbed to a microscopic slide, and 2 drops of 0.5 sodium dexoycholate are added. Try to make uniform suspension. Negative result is indicated by the formation of a uniform suspension that persist more than one minute. If the organism lyse so that little or any turbidity develops and the fluid becomes mucoid, such that a "string" is seen when the loop is slowly raised, the test is considered positive.

BIOTYPE OF VIBRIO CHOLERA

Most outbreaks of cholera are now caused by *V. cholera* biovar Eltor. In Bangladesh, however, cholera outbreaks due to Classical biovar.
The following tests can be used to distinguish the 2 biovars:

1. Voges-Proskauer test
2. Haemagglutination of chicken or sheep cells
3. Polymyxin B sensitivity test.

Test	Eltor	Classical
1. Voges-Proskauer	+	_
2. Heamagglutination test	+	_
3. Polymyxin B Sens.	Resistant	Sensitive

Serological Tests:

Vibrio species posses O and H antigens. *Vibrio cholera* is now divided into 84 serovars. Serovar O1 contains those strains that causes cholera and are agglutinated by *V. cholera* O group polyvalent antiserum.

There are subtypes of O1 group, they are differentiated based on the basis of specific antigenic structures.

Subtype	Anti-Inaba	Anti-Ogawa
Inaba	+	_
Ogawa	_	+
Hikogima	+	+

Treatment & Antibiotic Sensitivity:

The essential treatment of cholera consist of:

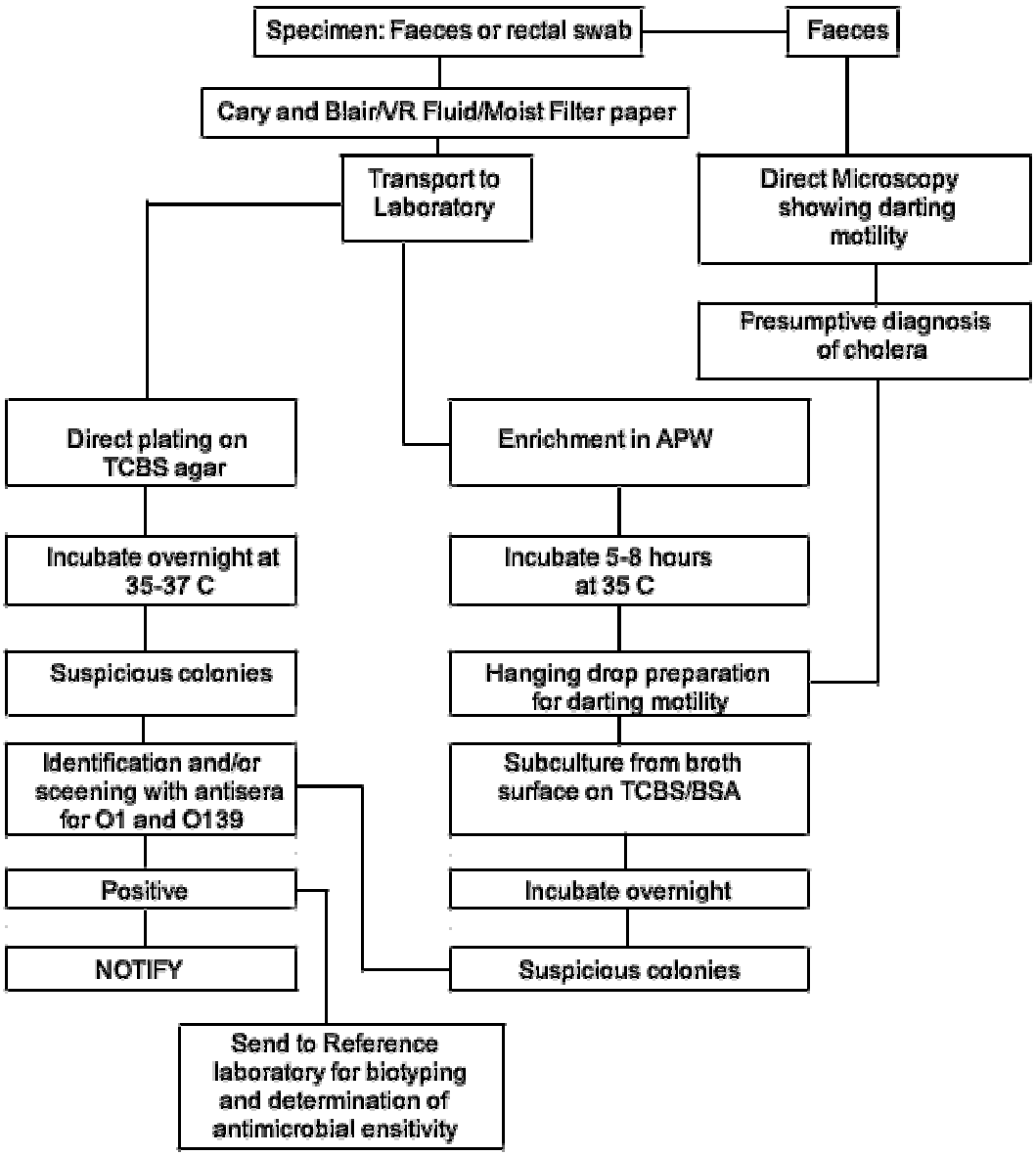
1. Fluid and electrolytes replacement
2. Antibiotic therapy to irradiate the organism.

Tetracycline is the drug of choice for the treatment of *V. cholera* infections. However, in recent years, tetracycline-resistant variants have emerged. For this, sensitivity tests must be performed on all clinical isolates. Among the known effective antibiotics; tetracycline group, sulfonamides, ampicillin, kanamycin, streptomycin and trimethoprim.

PREVENTION OF CHOLERA

- Good sanitation & personal hygiene
- Immunization with live-attenuated vaccine - stimulates local IgA production
- Chemoprophylaxis with tetracycline to close contacts
- Prompt detection & treatment of carriers

Chart 1: Flow Chart for Diagnosis of Cholera



CAMPYLOBACTER

SPECIES:

1. *Camplylobacter fetus subsp. fetus* Non pathogenic
2. *Campylobacter fetus subsp. intestinalis*
3. *Campylobacter fetus subsp. jejuni*
4. *Campylobacter pylori* or ***Helicobacter pylori*** (Peptic ulcer)

SOURCES (GIT & reproductive organs) of:
•Chicken, sheeps, cattles, dogs, cats etc.
•60% of chickens are contaminated

Direct occupational contact
Farmers, butchers & poultry processors

Direct domestic contact
Dogs & cats with campylobacter diarrhoea

Indirect transmission
Untreated water
Raw milk
Undercooked meat & poultry

Direct domestic contact
Children to children

DISEASES:

I. Disseminated infections:

1. meningitis and meiningoencephalitis in infants: Mortality rate is 50% despite intensive treatment. Specimens include blood, CSF and stool
Mostly caused by *C. intestinalis.*

2. Bacteremia: In children: caused by both *C. intestinalis* and *jejuni*. Specimen is blood.

3. Disseminated infections in adults: Usually in debilitated patients with one or more of the following conditions:
1. Cardiovascular disease

2. Hepatorenal
3. Endocrinological disorders
4. Chronic alcoholism
5. Malignancies

Causative organism: Mostly *C. intestinalis*

= Specimen: Blood, pericardium, pleura, joints and stool.

II. Enteric Infections:

Species of the genus campylobacter, are one of the major etiologic agents of bacterial enteritis. Campylobacter enteritis is accompanied by fever, headache, muscular pain, nausea and pain. 24 hours following this acute phase, diarrhea develops which may be bloody, mucoid and watery.

Isolation of Campylobacter from Stool:

1. Filtration Technique:

1. One gram of stool specimen is suspended in 20 ml saline
2. Agitate vigorously (on Vortex for 10-15 seconds).
3. Centrifuge at 650-800 rpm for 10 minutes.
4. Take four to five milliliters into a syringe
5. Pass the liquid through two 25 μm filter chamber:

=The upper non-sterile chamber is fitted with an 8.0 and 1.2 μm Millipore membrane filter.

= The lower steam-sterilized chamber contains a 0.65 *u*m membrane.

6. 2-4 drops of the filtrate are spread onto chocolate agar and incubated.
7. Both *C. jejuni* and *intestinalis* grow well on this medium and are very characteristics.

= Incubation conditions:

1. Microaerophilic environment: 5% O_2
10% CO_2
85% N_2

2. To increase the size of *C. jejuni*, incubate at 42 ^oC.

II. Selective media:

The introduction of selective media made the isolation of Campylobacter species from a stool sample possible:

EXAMPLE: Blood Agar + Antibiotic solution.

1. Skirrow solution	**2. Butzler**
=Vancomycin	=Bacitracin
=Polymyxin B	=Cyclohexamide
=Trimethoprim	=Colistine B sulfate
	=Cephazolin sodium
	=Novobiocin.

GENERAL CHARACTERISTICS OF CAMPYLOBACTER

= Small, delicate, spirally or curved.
= Gram-negative bacteria
= Oxidase positive
= Fastidious
= Microaerophilic
= Motile by polar flagella

Advantages of filtration technique over the selective media

1. All non-campylobacter organisms are retained by the filters.
2. Both *C. intestinalis* and *jejuni* will grow on Chocolate agar unlike the selective media which allows only one of the two.
3. You do not need to worry about the decay of antibiotics as in the selective media.

Differential Reactions & Characteristics of Species of the Genus Campylobacter

Species	Catalase	Nitrate	H_2S	Urease	Growth at				
					25	37	42	N	C
1. *C. fetus*	+	+	-	-	+	+	-	R	S
2. *C. intestinalis*	+	+	+	-	(+)	+	-	R	S
3. *C. jejuni*	+	+	-	-	-	+	+	R	S
4. *H. pylori*	+	V	+	+	-	+	+	R	S

(+) Most strains are positive
N = Nalidixic acid
C= Cephalothin

SEROLOGICAL TESTS:

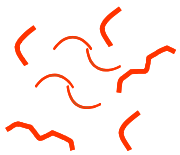
Serological tests are not suitable for routine investigation due to the lack of standardized antigen suspensions and reference sera.

ANTIBIOTIC SENSITIVITY:

Erythromycin is the drug of choice. Chloramphenicol, aminoglycosides, carbenicillin, clindamycin and tetracycline are also effective.

HELICOBACTER PYLORI

- Previous name : Campylobacter pylori
- First isolated in 1983 from human stomach
- Gram-negative bacilli
- Curved, spiral or seagull-shaped
- Motile with multiple polar flagella



CULTURAL CHARACTERISTICS

- Grow best at 42-43 ^{o}C
- Microaerophile (7% oxygen)

- Strongly urease positive (different from Campylobacter)
- Grow on enriched & selective media Modified Skirrow's agar (Blood agar with antibiotics)

HABITAT

- Human gastric mucosa (world-wide distribution)
- Approx. 50% of adults >60 years are infected

TRANSMISSION (person to person)

- Oral-oral
- Fecal-oral
- There are clusters of infection in families

DISEASES BY *H. PYLORI*

- Gastritis
- Gastric & peptic ulcer
- Gastric cancer
- Most individuals tolerate the presence of H. pylori for decades

CLINICAL FEATURES

- Incubation period : few days
- Nausea, flatulence & bad breath
- Recurrent epigastric pain & dyspepsia
- Epigastric burning sensation
- Bleeding from ulcer
- **No dissemination**

TREATMENT

Triple therapy

- Metronidazole +
- Clarythromycin or amoxycillin +
- Omeprazole

Vaccine development : under trial

LAB IDENTIFICATION

Specimen : gastric biopsy
Direct gram-stained smear of crushed biopsy
Direct urease test
Place a piece of biopsy in urea broth- red color change in few min to 2 hrs

- **Culture : On Skirrow's medium**
  - Oxidase +ve, Urease +ve, catalase +v

- **Serodiagnosis**
  - ELISA to detect IgG (not established to differentiate active vs past infection)

° *H. pylori* Stool Antigen (HpSA) test

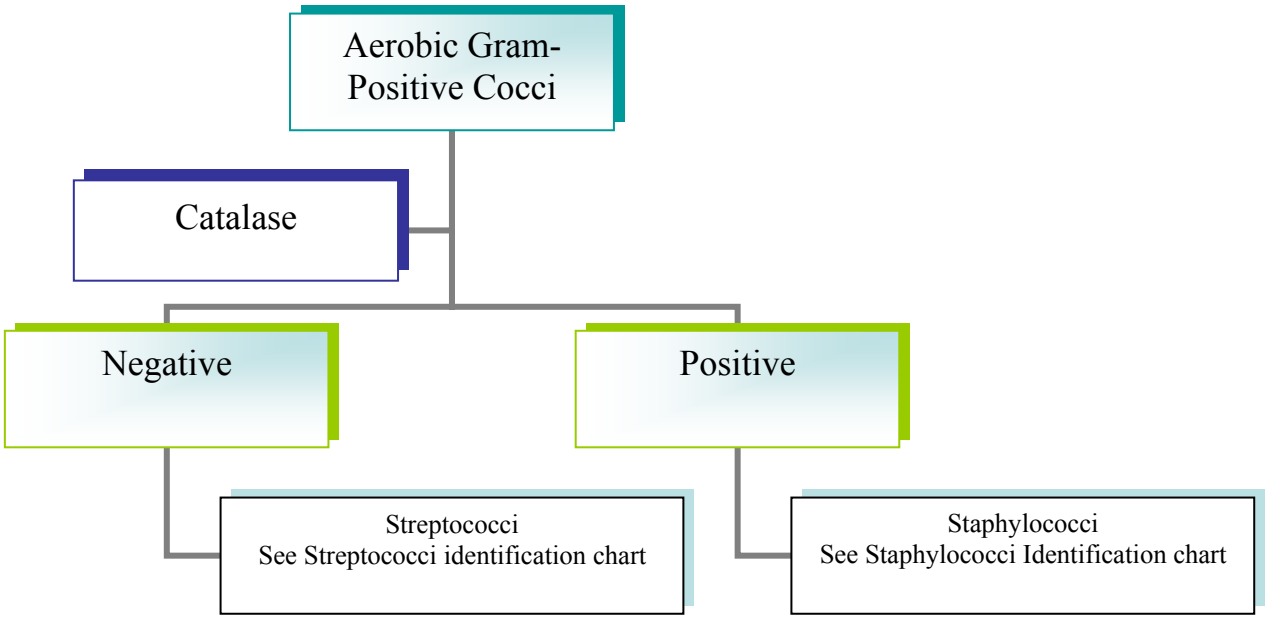
Urea Breath Test

- Patient ingests radio-labeled ^{14}C urea

If *H. pylori* infection present :

- Urease produced by the organism, hydrolyses urea to NH_3 and radio-labeled $^{-}HCO_3$ that is exhaled as CO_2 which is detected by spectrometer
- Has good sensitivity & specificity.

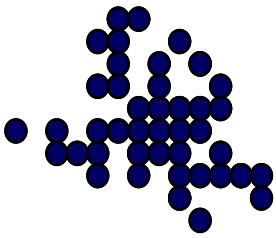
THE GRAM POSITIVE COCCI



THE STAPHYLOCOCCI

Staphyle (Greek)	Bunch of grapes
Cocci	Berries

SPECIES:



33 species are known. Three are medically important:

1. *Staphylococcus aureus* Most important pathogen
2. *Staphylococcus epidermidis* May cause endocarditis
3. *Staphylococcus saprophyticus* May cause cystitis.

General Characteristics:

1. Cocci arranged in grape-like clusters
2. Strongly gram-positive
3. Ferments many carbohydrates with the production of lactic acid but no gas
4. Non-motile
5. Non-spore forming

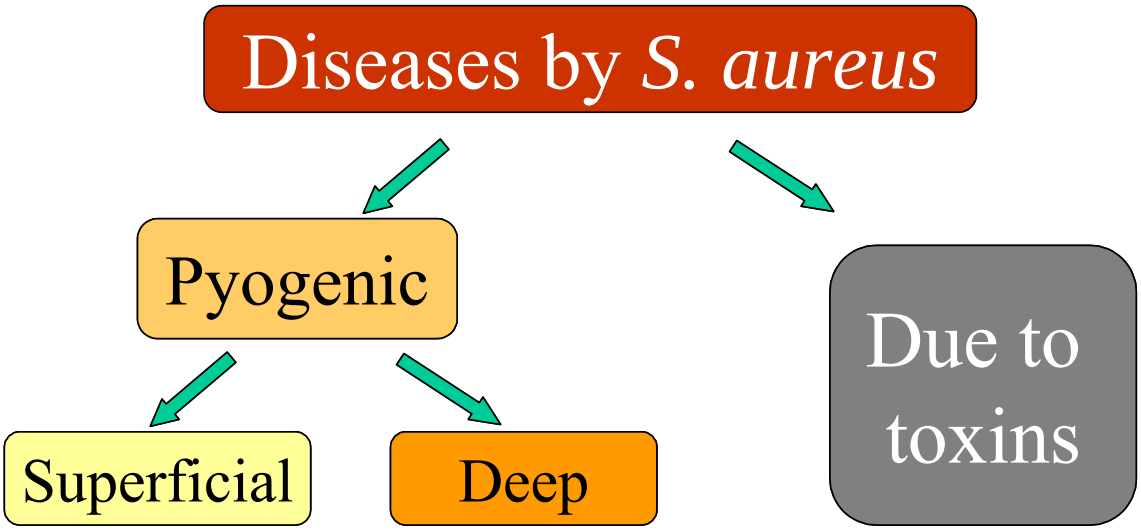
Staphylococcus aureus

Aureus: golden color (golden color colonies on blood agar)

Diseases caused by the organism:

1. Toxic shock syndrome	**2. Furuncles (abscess)**
2. Septicemia	**4. Impetigo (minor skin infection)**
5. meningitis	**6. Pneumonia**
7. Food poisoning	**8. Pyoderma**

9. Carbuncles



Cultural and Morphological Characteristics:

1. Media for Primary Isolation: *S. aureus* grow well in most routine media e.g, Blood Agar, Nutrient Agar.

2. Media for Selective Isolation: Mannitol Salt Agar (MSA), is an excellent medium which contains 7.5% sodium chloride which is considered as a high percentage and inhibitory to most medically important bacteria. In addition it contains mannitol as the only carbon source and a pH indicator to detect mannitol fermentation by *S. aureus.*

3. Incubation: After streaking the specimen on one of the common media, incubate the plates at 35-37 ºC for 24 hours.

4. Colony Morphology:
4.1 On blood agar plates: colonies are 2-4 mm in diameter, rounded and slightly elevated. Most pathogenic strains produces a zone of β-hemolysis. Another distinguishing character is the production of a **golden yellow pigment**.

4.2 On Mannitol Salt Agar: The colonies are surrounded by a yellow zone indicative of acid production resulting from the fermentation of Mannitol.

5. Gram Stained Smears:
Gram-positive cocci arranged in clusters. Single cells, diplococci, and short chains may also appear. It is usually simple to identify the morphology in stained film from sputum or pus but one can be certain by performing simple biochemical tests for the isolate e.g., **catalase test** to differentiate it from Streptococci and **Coagulase** or **DNase** to differentiate it from non-pathogenic staphylococci..

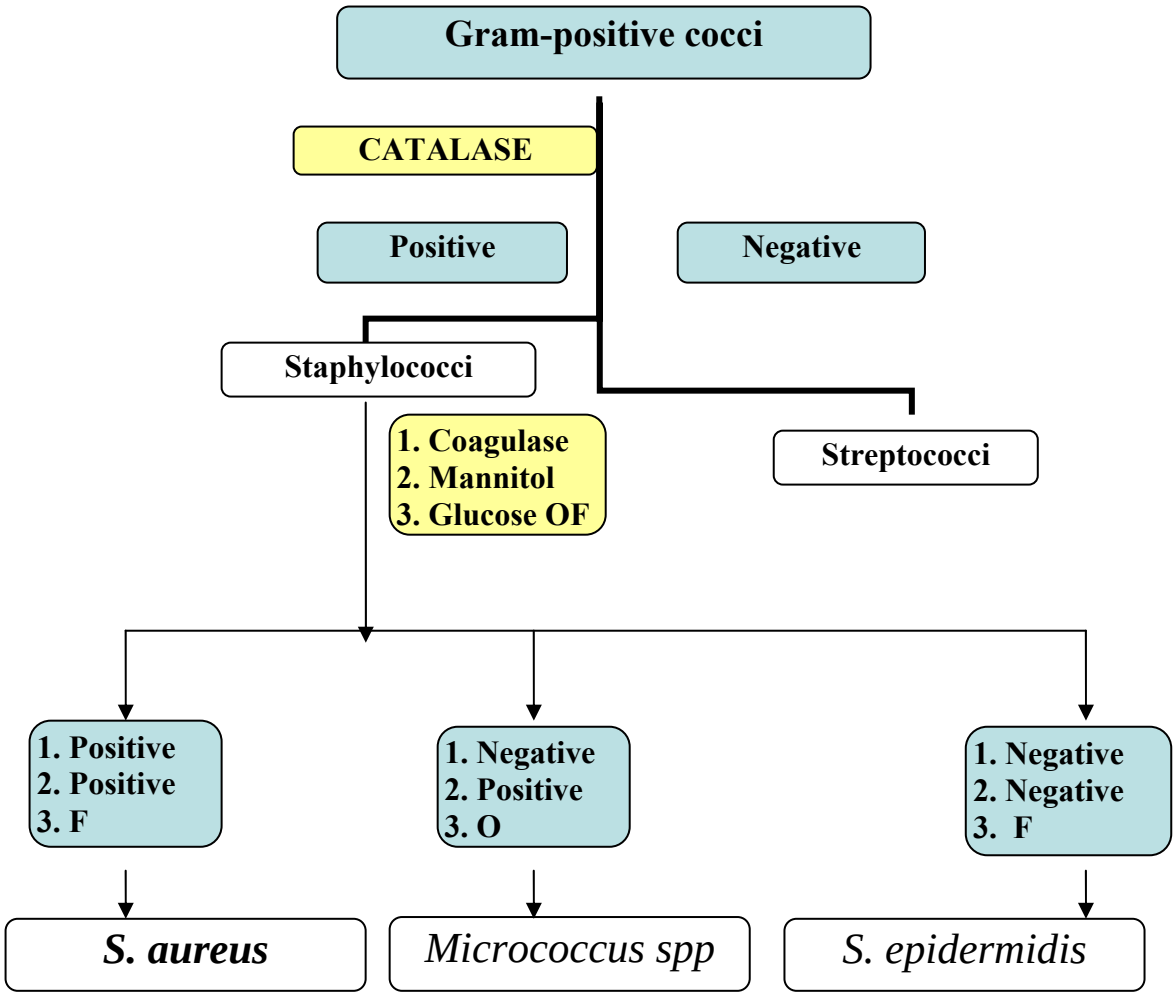
6. Biochemical Characteristics:
1. Catalase positive
2. Mannitol fermenter
3. Grow well in 7.5% NaCl
4. Coagulase positive
5. DNase positive

6. Glucose fermenter.

IDENTIFICATION:

1. Based on gram-staining
2. To differentiate it from other gram-positive cocci (Streptococci) perform catalase test
3. To differentiate it from other non-pathogenic staphylococci a group of biochemical tests are performed.

a. Coagulase test
b. Mannitol fermentation
c. Growth in 7.5% NaCl
d. Glucose OF
e. DNase



NB: For epidemiological reasons, phage typing of *S. aureus* is necessary.

PHAGE TYPING:

A *Staphylococcus aureus*-specific phage is added to a plate that is inoculated with *S. aureus*. The plates are incubated at 37 ^{o}C for 24 hours.

Positive identification: Formation of plaques

Negative results: *S. aureus* grow over the whole area.

SENSITIVITY TESTING AND TREATMENT:

*S. aureus i*s a frequent hospital pathogen and it has the ability to develop resistance to the commonly known antibiotics. For this reason sensitivity testing must be performed on all isolates. **Penicillin G** and its derivatives (**ampicillin**, **amoxycillin**, **cloxacillin**, **methicillin**), **ofloxacillin** and **cephalosporins** are usually effective against *S. aureus.*

TREATMENT OF STAPH INFECTIONS

- Drainage of pus in superficial and chronic lesions
- C/S to select proper antibiotics

Infections	Treatment
a) Severe infections	PRP* (cloxacillin, methicillin)
b) Due to MRSA (methicillin-resistant S. aureus)	Vancomycin (Final choice)
c) Chronic infections	PRP (given for months)

*PRP : Penicillinase resistant penicillins

ANTIBIOTICS RESISTANCE

Historical aspect

- 1940s : all *S. aureus* were sensitive to penicillin
- Shortly after use : penicillin resistant strains appeared which produced beta-lactamase - rapidly spread
- In late 1950s : beta-lactamase - resistant penicillin (methicillin) (not degraded by)
- In 1961 methicillin-resistant *S. aureus* (MRSA) was discovered (presently a major problem)

EPIDEMIOLOGY OF STAPH INFECTIONS

Source of Infection (Carrier)

- Anterior nose of 20-40% of adults
- Physicians & nurses = 50-70%
- Skin of axillae & perineum
- In hospital - high due to environmental load

MRSA
• Low carriage rate in community
• High in tertiary care hospitals
Mode of Transmission
• Fomites
• Direct from hospital staff or attendants : contaminated hands

PREVENTION OF STAPH INFECTIONS
Control of Carrier and reinfection
• Wash clothes in hot water (>70^{o}C)
• Use antiseptic soap (Dettol soap)
• Antimicrobial nasal cream (Gentamicin, Mupirocin)
• Oral antibiotics that are concentrated in nasal secretions (ciprofloxacin and rifampicin)
Chemoprophylaxis
• Antibiotics before and at time of surgical operation

COAGULASE-NEGATIVE STAPHYLOCOCCI (CNS)

- **Medically important species:**
  1. *S. epidermidis*
  2. *S. saprophyticus*

Staphylococcus epidermidis

• Normal flora in
  ° Skin,
  ° Anterior nose &
  ° External ear canal
• Cell wall contains teichoic acid (glycerol type)
• White, non-haemolytic colonies on blood agar
• Sensitive to novobiocin; (S. saprophyticus is resistant)

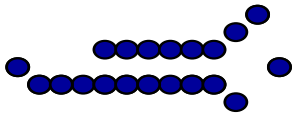
DISEASES BY S. EPIDERMIDIS
• Most infections are hospital acquired
• Opportunistic pathogen in immuno-suppressed
• Strongly associated with presence of foreign bodies
° Prosthetic heart valves (endocarditis)
° IV catheters (bacteremia)
° Urinary catheter (UTI in elderly)
° CSF shunts (meningitis)
° Peritoneal dialysis catheter (peritonitis)

Staphylococus saprophyticus

• Saprophytic life
• Resistant to novobiocin
• Most infections are community-acquired
° Primary UTI in 10-20% of young adult women – hormonal factors may be involved.
• Resistant to antibiotics – penicillins & cephalosporins

THE GRAM POSITIVE COCCI
THE STREPTOCOCCI

PATHOGENIC SPECIES:



1. *Streptococcus pyogenes*
2. *Streptococcus agalactia*
3. *Streptococcus faecalis*
4. *Streptococcus pneumonia*
5. The viridans group (*Streptococcus viridans*)

General Characteristics:

1. Gram positive cocci arranged in chains
2. Catalse Negative
3. Colonies are small (1-2 mm), gray to white in color
4. Streptococci could be classified based on their heomlytic activity into:

A. Alpha hemolytic: That produces incomplete hemolysis on blood agar as indicated by greenish discoloration of the medium (Greenish zone around colonies due to degradation of haemoglobin to biliverdin (green) ,e.g., *S. pneumonia* and *Viridans streptococci*

B. Beta hemolytic: That produces complete hemolysis which results in clear zone around the colonies. e.g., *S. pyogenes* and *S. agalactiae*

C. Gamma hemolytic (Non hemolytic): That produces no hemolysis on blood agar plates. e.g., *S. faecalis* (*Enterococcus faecalis*)

5. Lancefield grouping (on the basis of C-carbohydrate antigen in cell wall of streptococci)

• Groups A-U (group A,B & D are of great pathogenic significance.
° Group A = *S. pyogenes*
° Group B = *S. agalactiae*
° Group D = *Enterococci*
• Basically beta-haemolytic are grouped by this method
• Some alpha and non-haemolytic streptococci contain Lancefield group antigens

NB: Many textbooks use a combination of the Lancefield and the hemolysis classification. e.g., group A Beta hemolytic streptococci

Streptococcus pyogenes
Group A beta-hemolytic Streptococci

Diseases:

1. Erysipelas (red skin)	**7. Streptococcal sore throat**
2. Otitis media	**8. Osteomyelitis**
3. Puerperal fever	**9. Endocarditis**
4. Wound infection	**10. Meningitis**
5. Sepsis (Scarlet fever)	**11. Acute glomerulnephritis**
6. Sinusitis	**12. Rheumatic fever**

NB: Streptococci causes a wider variety of clinical infections than any other genus of bacteria.

Specimens for Culture:

1. Throat swab: A tongue depressor should be used to expose the posterior pharynx. A sterile swab is then rubbed firmly over the tonsil and posterior pharynx, touching any exudates present.

2. Nasal swab: May be obtained by swabbing the anterior nares at a depth of 1-2 cm with a cotton swab.

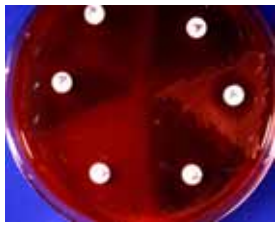
3. Sputum culture: Are useful for detecting lung infection due to pneumococci and other streptococci. Sputum usually is contaminated with saliva containing normal flora, hence, special precaution should be observed. Specimens obtained by transtracheal aspiration are more reliable.

4. Blood: Blood cultures should be obtained from all patients with possible bacteremia or endocarditis.

DIAGNOSTIC PROCEDURES:

1. Gram stain: The presence of infections involving streptococci can frequently be determined both quickly and easily by microscopic examination of gram stained smears of certain types of specimens.

2. Culture of specimen: Blood agar plates are the most commonly used for primary isolation of streptococci. In mixed infections: Selective media should be used such as blood agar containing **gentamicin, colistin and nalidixic acid**.
= Colony morphology is not a distinguishing characteristic.



3. Biochemical and Other tests:

1. **Catalase test** (negative)

2. **Bacitracin sensitivity test:**

a. Select a colony from the culture to be tested; streak it evenly over the surface of a half blood agar plate.

b. Place bacitracin disk in the center of the streaked area.

c. Incubate overnight

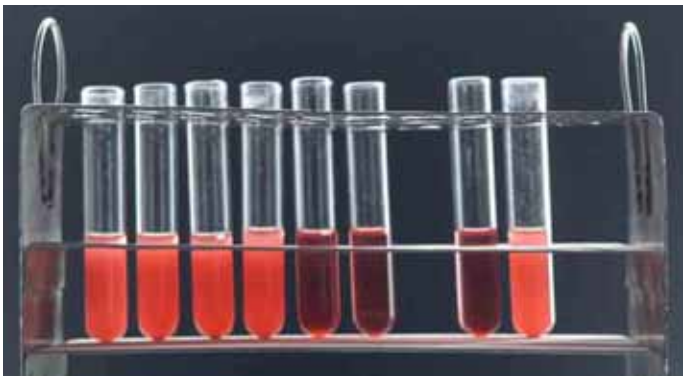
d. **RESULT**: An inhibition zone of 5mm or greater is considered sensitive.

NB: *Streptococcus pyogenes* is sensitive to bacitracin

Serological tests:

Detection of antibody in patient serum

ASOT: Anti-Streptolysin O Titer: Streptolysin O is an antigen carried on the cell wall of *S. pyogenes* that is usually anticipated by specific antibodies produced by the host. This test is simple and reliable. The test is done by performing a serum serial dilutions and reacting these dilutions with the specific antigen suspension and determining the lowest concentration or the highest dilution **(Titer)** that produces a positive results.



Treatment:

Penicillin G is used in the treatment. In penicillin sensitive patients **Erythromycin** is used.

Streptococcus agalactia
Group B, beta-hemolytic streptococci

General Characteristic:

- It is the only species that represents the group Streptococci
- Hemolysis on Sheep Blood Agar is mostly beta-hemolysis
- Catalase negative
- The group antigen is a cell wall polysaccharide composed of N-acetylglucoseamine, galactose, and rhamanose.
- It hydrolysis hippurate to benzoic acid and glycine.
- It is resistant to bacitracin antibiotic disk (and could be differentiated from *S. pyogenes* which is a beta-hemolytic and sensitive to this antibiotic).

Normal Human Habitat:

It is a part of the normal oral and vaginal flora. Approximately **5-15% of the healthy population carry *S. pyogenes* or *S. agalactia* in the nasopharynx**. It can be found in pharynx, vagina, gastrointestinal tract. In addition, in the newborn it could be present in various sites.

Pathogenicity:

1. Puerperal sepsis
2. Endocarditis
3. Pneumonia
4. Neonatal infection (Pneumonia, septicemia and meningitis).
5. Bovine mastitis

Culture of the Organism:

1. On general media: e.g., Blood Agar; *S. agalactia* produces larger colonies and more translucent to opaque colonies surrounded by a zone of Beta-hemolysis.

2. Selective media: (For both *S. pyogenes* and *S. agalactia*): Streptococcal Selective Agar (SSA): Contains the following inhibitory chemicals:

- Crystal violet in low concentration,
- Colistin,
- Trimethoprim-sulphamethoxazole in 5% sheep blood agar.

Laboratory Diagnosis:
1. Lancefield grouping
2. Hippurate hydrolysis test
3. CAMP test
4. Bile Esculin test
5. Latex agglutination.

NOTE: The sodium hippurate hydrolysis and CAMP tests can be used to confirm the identification of group B streptococci.

1. Hippurate hydrolysis test:
Group B streptococci contains the enzyme hippuricase which can hydrolyze hippuric acid. The products of the hydrolysis of sodium hippurate are sodium benzoate and glycine. Glycine could be detected by the addition of ninhydrin which is an oxidizing agent, which alos gives a purple color with glycine.

2. CAMP test
The hemolytic activity of Staphylococcal Beta lysin on RBCs is increased by an extracellular factor produced by *S. agalactia* called the CAMP factor.

This test is don by making a single streak of Streptococcus (to be identified) on sheep blood agar perpendicular to a strain of *Staphylococcus aureus* known to produce beta-lysin. The two streak lines must not touch one another. The plate is incubated for 24 hours . The positive result is expressed by a zone of increased lysis assuming the shape of an arrow-head at the junction of the two streak lines.

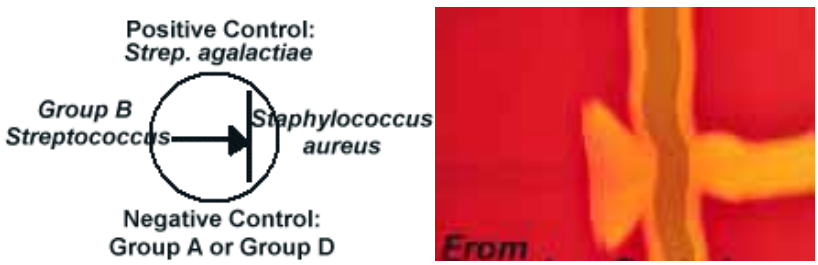


Fig. Showing the arrow-head appearance of a positive strain

3. Bile Esculin
This test detects the ability of the organism to grow in the presence of bile and its ability to hydrolyze esculin and the production of glucose and aglycone esculetin. Esculetin reacts with iron salts to for a dark brown or black complex. This test is performed in an appropriate medium containing bile, esculine, ferric citrate as a source of ferric ions, and sodium azide to inhibit the growth of gram negative bacteria. This test used to differentiate **Group D streptococci (Enterococci) POSITIVE**, and ***Streptococcus agalactia* (NEGATIVE)**

3. Latex agglutination:

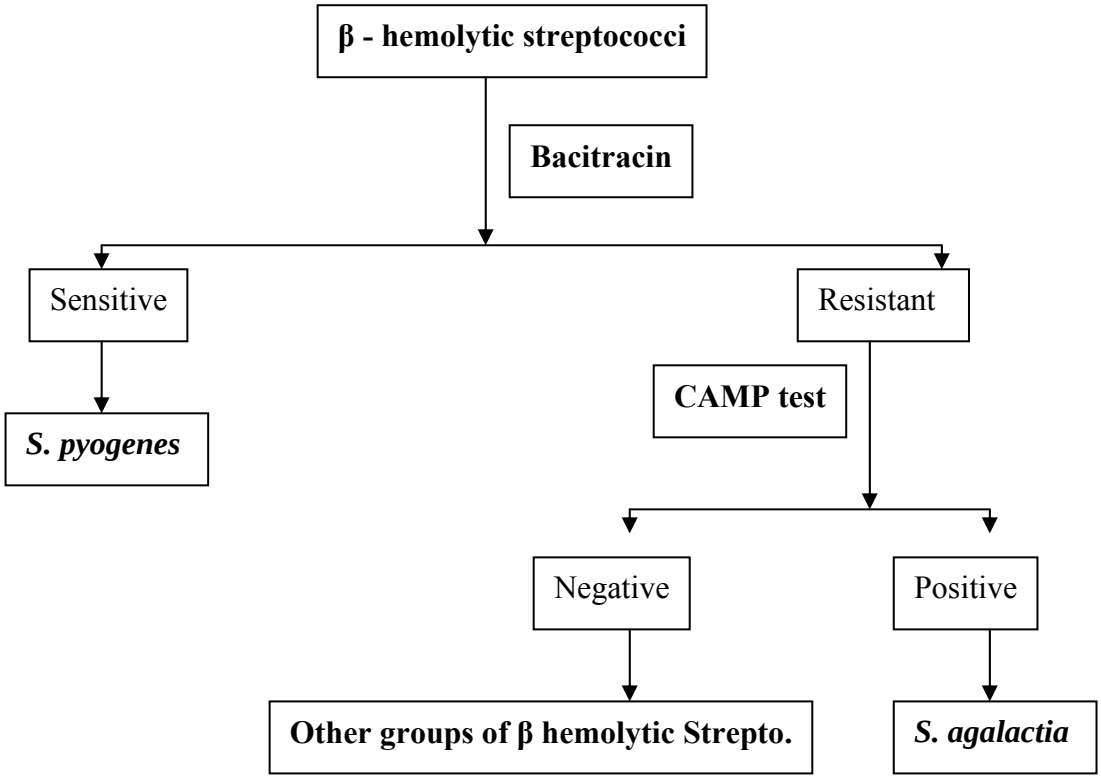
A. Antibody coated latex particles serves as the basis for several commercially available systems for direct detection of bacterial and other microbial antigens in body fluids. For example, Streptococcal antigens in throat swab samples can be now detected within 10-60 minutes depending in the system being used.

B. Latex agglutination tests are also available to detect antibodies that develops during certain bacterial infections.

The advantages of the latex agglutination tests is rapidity and its relative sensitivity.

Treatment:

Group B streptococci is often resistant to tetracycline and kanamycin but remain sensitive to the clinically achievable blood levels of penicillin G.



Streptococcus pneumoniae

This species was isolated by Pasteur in France in 1881 and was found to be the major cause of lobar pneumonia in human. Two variants has been isolated; one with capsule and was demonstrated to be pathogenic for both man an mice and the other is non-capsulated was found to be non-pathogenic.

Normal Habitat:
It is normally found as an oral flora and in the nasopharynx. In general 15% of children and 5% of adult healthy individuals whose considered to be carriers, do not suffer pneumonia.

Diseases:
1.Pneumonia (Pneumoncoccal pneumonia): This type of pneumonia usually occur most frequently with the following conditions:
a. Viral infections of the upper respiratory tract.
b. Persons whose respiratory drainage is impaired e.g., heavy smokers and persons who have inhaled toxic irritants
c. Immunocompramized patients
2. Bronchitis
3. Sinusitis
4. Otitis media
5. Septicemia
6. Meningitis

Antigenic Structure:
1. Capsular Antigen : The capsular polysaccharide is highly antigenic and one could classify *S. pneumoniae* into 84 serotypes. Capsule is considered to be the major virulence factor by which it resist the process of phagocytosis by PMNLs and macrophages.
2. Somatic Antigen (O): C polysaccharide in the cell wall comosed of teichoic acid polymer. This antigen react with Beta-globulin refered to as C-Reactive Protein (CRP).
3. M protein (Somatic): protein which neither antiphagocytic no protective.

Virulence:
1. Polysaccharide capsule
2. Adherence
3.Enzymes
- **a. Neuroaminidase**: Degrades surface structures of the host tissue
- **b. Protease**: degrades immunoglobulin

4. Pneumlysin *O*: Oxygen labile toxin which binds to cholesterol molecule in the host cell membrane and lyses the cell.
5. Autolysin: Lysis the cell in the presence of surface active substance such as bile salt. This lysis facilitate the release of pneumolysin and other toxic materials from the cells.

Culture Media:

- Blood agar is usually an excellent medium for the primary isolation of this organism. Chocolate agar is also used.
- 5-10% CO_2 enhances growth (CO_2 incubator). In order to visualize alpha hemolysis, stab the inoculating loop into the agar several times to allow the action of the oxygen labile streptolysin to take place.
- Capsulated strains; on blood agar, produces white circular mucoid colonies surrounded by a zone of alpha hemolysis as indicated by a greenish discoloration.

Gram staining:

-Gram-positive diplococci (lancet-shape). May show gram negative reaction due to the action of antibiotics or autolysin and especially in old cultures.

Laboratory Diagnosis:

- Gram stained smears from clinical specimen and from culture should revail the typical lancet shaped gram positive diplococci.
- Biochemical & Serological Identification:

1. Quellung reaction:

The reaction is performed by mixing equal amounts of specimen with type specific pneumococcal antiserum and waiting 15-30 minutes to allow the reaction to occur, then examine by the oil immersion objective. This preparation is compared with a saline perpetration to detect any swelling (Enlargement) of the capsule.

2. Capsule Negative Staining: Capsule could be demonstrated by staining cultures by INDIA INK.

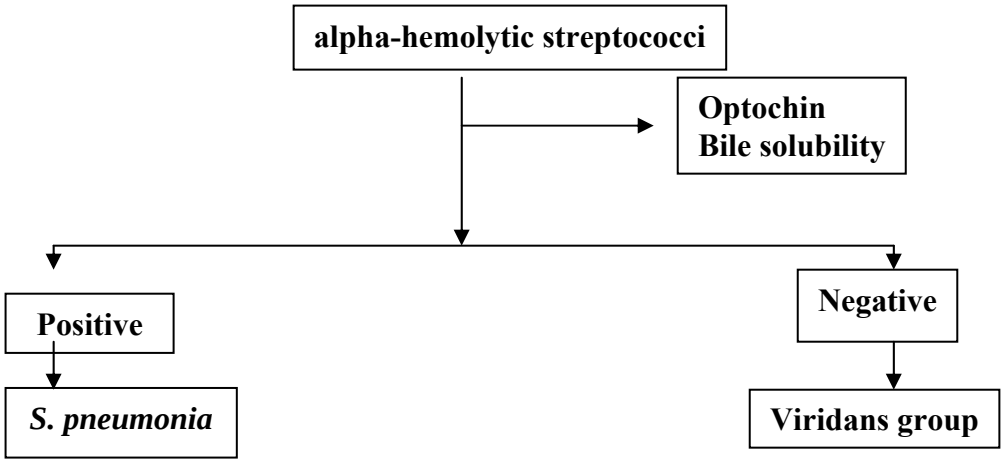
3. Catalase test = Negative

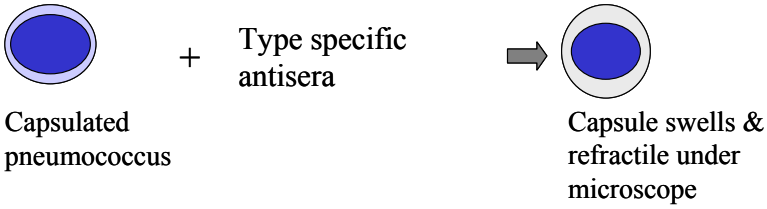
4. Bile solubility test: 10% sodium deoxycholates lyse colonies of *S. pneumoniae*. This test is done by adding a small drop of the bile salt over suspected colony and is observed for lysis.

5. Optochin susceptibility test:

Optochin has a detergent-like action and cause selective lysis of pneumococci. Zone of inhibition of 14 mm (6 mm disks). This test should be performed in 5-10% CO2.

6. Detection of pneumococci antibodies: By the radioimmunoassay which is very sensitive in detecting the specific capsular antibodies.





CAPSULAR SWELLING (QUELLUNG) REACTION

- Capsulated pneumococci are mixed with type specific antisera
- The capsule absorbs water and swells
- Refractile under light microscope
- Polyvalent antiserum containing antibodies against 80 capsular types is available

GROUP D STREPTOCOCCI

Non-haemolytic or alpha-haemolytic colonies: Two types

1. Enterococci (*E. faecalis*)

- Normal flora of colon
- Can grow in presence of 7.5% NaCl and bile salts

Diseases

- Opportunistic UTI
- Wound infections

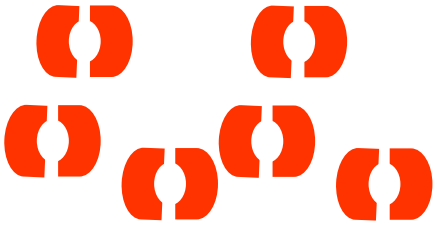
2. Non-Enterococci (*S. bovis*)

- Can grow in presence of bile salts (not NaCl)
- May cause endocarditis

THE GRAM NEGATIVE COCCI

SPECIES:

1. Neisseria gonorrhea
2. Neisseria meningitidis
3. Neisseria catarahlis
4. Neisseria sicca
5. Neisseria flavescens
6. Neisseria flava
7. Neisseria mucosa
8. Neisseria lacatamica



1. Neisseria gonorrhea

The name gonorrhea was introduced by Galen 130 A.D. Neiser 1879 described diplococci which he found in the purulent secretions of acute cases of urethritis and vaginitis and in smears made from acute conjunctivitis of the new born. Gonorrhea is the most prevalent venereal disease and is second only to tuberculosis among infectious diseases which give rise to serious complications. The primary infection with *N. gonorrhea* although uncomfortable gives no alarming symptoms and too frequently ignored by the patient. Man is the only known host for the organism.

MORPHOLOGY AND STAINING

Coffee-bean shaped, kidney-bean shaped, in pairs, gram-negative diplococci. In smears form urethral discharges, the gonococcus is seen as an oval; or spherical coccus. Found frequently in pairs with the adjacent sides flattened. Usually intracellular, especially in acute cases. In smears there is irregularity in the distribution in the phagocytic cells, many polymorphonuclears containing no organism while a few cells may contain as many as 20 to 50 or more cocci per cell.

In acute cases, intracellular cells predominate over the extracellular forms while in chronic cases , the extracellular form predominate over the intracellular forms.

Non-motile, nonencapsulated except in its mucoid variant phase. Stain readily with aniline dyes.

The presence of gram-negative intracellular diplococci in smears of pus from the male urethra is presumptive evidence of gonorrhea.

CULTURAL CHARACTERISTICS: In exudates from the vagina or from the eye, however, the morphologic picture is less reliable since the other gram negative cocci present frequently in these regions.

Delicate aerobes which usually needs an atmosphere containing 5-10% CO_2 and an optimum temperature of 35-36 ^{o}C. The pH of the medium should be adjusted to 7.2 to 7.6.

MEDIA OF CHOICE:

1.CHOCOLATE AGAR

2.TRANSGROW: A transport medium to maintain viability of the organism during transportation.

3. Thyer-Martin Medium: Very similar to the chocolate agar to which antibiotics has been added:

a. Vancomycin: To inhibit the growth of gram-positive cocci.

b. Colistine: To inhibit growth of gram negative organism

c. Nystatin: To inhibit growth of fungi.

Trimethoprim may be added to prevent the growth of swarming organisms.

On Chocolate agar, after 48 hours of incubation, round, convex smooth grayish colonies appear. On further incubation, the colonies may increase in size and develop a roughened surface with crenated edges. The colonies are soft and somewhat slimy when touched by a platinum loop.

In broth medium, the growth is chiefly on the surface with some flaky sediment at the bottom of the tube. Dextrose is fermented producing acid but not gas.

On Thayer-Martin Agar (Medium of choice): After 20 hours of incubation at 35-36 ^{o}C in a candle jar, typical colonies appear small translucent, raised, moist gray white colonies with entire to lobate margins. Usually they are mucoid and tends to come off as whole colonies when fished from the agar surface.

FERMENTATION REACTIONS OF NEISSERIA

Organism	Dextrose	Maltose	Sucrose	Growth on NA	Growth at 37 ^{o}C	oxidase
N. meningitidis	+	+	-	-	-	+
N. gonorrhea	+	-	-	-	-	+
N. catarrhalis	-	-	-	+	+	+
N. sicca	+	+	+	+	+	+
N. flavescens	-	-	-	+	+	+

PATHOGENICITY:

1. GONORRHEA: This infection is transmitted directly from individual to individual. It attacks chiefly the urethra of both males and females.The difficulty in growing gonorrhea in the laboratory as well as their failure to infect laboratory animals, makes it clear how very closely these organisms are adapted to growth in the human body. They will survive only for brief periods outside the body. This is the reason why gonorrhea is almost always transmitted by direct body contact, and principally by sexual intercourse.

Gonorrhea is a venereal disease accompanied by excessive purulent discharge from the genital tract. Incubation period is 3-5 days. if left untreated it may cause sterility in both males and females. Manifestation are urethritis, cervico-vaginitis, rectal involvement, pharyngeal involvement.

2. OPTHALMIA NEONATORUM

Less frequent site of primary infection is in the conjuctiva, usually seen in the newborn babies. This conjunctivitis in the newborn which result from infection during

birth when the fetus passes through the birth canal. If untreated this may lead to blindness. This disease could be prevented by the application of 2% silver nitrate solution in the eyes of all newborn.

3. PELVIC INFLAMMATORY DISEASE (PID)

DIAGNOSIS:

1.Specimen: Vaginal swab and material obtained from males by prostatic massage or urethral swab.

2.Stained smears: Smears from suspected cases will reveal gram negative diplococci, coffee-bean shaped.

Recovery of gram-negative diplococci extracellular or intracellular may be of a diagnostic value for male patient but usually are not in case the patient is female. This must be confirmed by culture and biochemical identification of the species.

3.Cultivation: on chocolate agar or Thayer-Martin agar with increased CO_2 tension at a 35 ^oC.

4. Oxidase Reaction: This depends on the formation of indophenol oxidase, which is common to all Neisseria.

5. Confirmatory tests:

A. Fluorescent antibody tagging test: (FAT)

B. Degradation of carbohydrates. The recommended base medium is **Cystine Trypticase Agar (CTA).** *Neisseria gonorrhea* ferments **only glucose** with acid but no gas.

RESISTANCE:

Easily killed by drying for 2 hours. Moist heat kills them at 55^oC in less than 5 minutes. Very susceptible to antiseptics, especially silver nitrate which kills the gonococci in 2 minutes using 1:4000 dilution. Cultures maintained at room temperature dies in 1 to 2 days and after 4 to 6 days at 36 ^oC.

Gonococci are susceptible to sulfonamides and penicillines although resistant strains to those antibiotics has emerged. Sensitivity tests must be performed on all isolates to determine their susceptibility.

ANTIGENIC STRUCTURE/ DETERMEINANTS OF PATHOGENICITY

1. Pilus antigen
2. Lipoligosccharide (LOS)
3. Outer membrane proteins constituents

P I. Antigenically variable and used as the basis of Enzyme-linked immunosorbent assay (ELISA) and coagulation assay for serotyping gonococci.

P II. Associated with adhesion properties.

NEISSERIA MENINGITIDIS MENINGOCOCCI

Neisseria meningitidis causes dramatic and explosive epidemics of cerebrospinal meningitis. Man is the only natural host. The organism live in the nasopharynx of apparently healthy individuals and are passed from man to man via reparatory tract.

MORPHOLOGY AND STAINING:

In cerebrospinal fluid (CSF) the organism appear as intracellular diplococci and sometimes in tetrads or even in large aggregates. In the early stages of the disease many of the organisms are found extracellularly. Both intra and extracellular forms are flattened on their opposite sides and resembles pairs of coffee-beans. Non-motile, non-sporeformers. Most of the isolated strains of Group I and Group II type have capsules. Gram negative

CULTURAL CHARACTERSTICS:

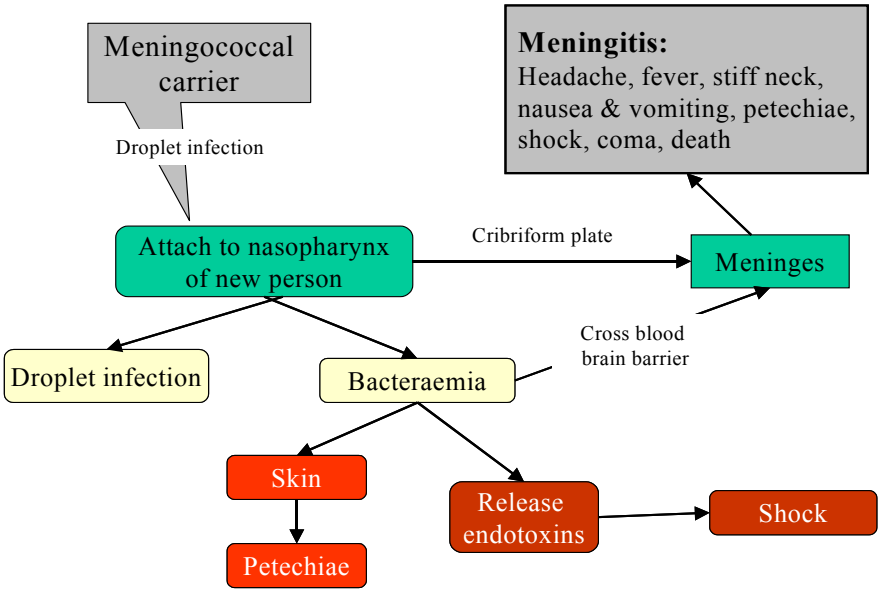
Rich medium with blood, serum, ascitic fluid and dextrose favors the growth.
In blood agar plates: Small glistening, slightly convex colonies are formed. Growth occur at aerobic conditions at 37 ^{o}C and 5-10% CO_2.

PATHOGENICITY:

= Causes epidemics meningococcal meningitis or cerebrospinal fever.
= this disease is transmitted through the inhalation of air borne droplets or by close contact with carriers.
Invasion of the body occurs in three steps:

1. Implantation of meningococcus in the nasopharynx.
2. Entrance to the blood stream with resulting septicemia.
3. Locoalization in the meninges.

Pathogenesis of meningococcal meningitis



The nasopharynx is the portal of entry of this organism. There, the organism may form part of the transient flora without producing or may produce an exudative pharyngitis. From the nasopharynx, organisms may reach the blood stream, producing bacteremia (meningococcemia) with high fever and a hemorrhagic rash. There may be fulminant sepsis, disseminated intravascular coagulation, and circulatory collapse.

Meningitis is the commonest complication of meningococcemia. It usually begins very suddenly, with intense headache, vomiting, and stiff neck and progress to coma within a few hours.

VIRULENCE FACTORS:

Lipopolysaccharide endotoxin complex which activates the clotting cascade producing disseminated intravascular coagulation.

IMMUNITY:

Moderate degree of immunity follows an attack of meningitis. Immunity increases with age because the disease is more prevalent in children than in adults. Infants have passive immunity through IgG antibodies transferred from the mothers.

ANTIGENIC STRUCTURE AND GROUPING

- Nine groups (A,B,C,D,E, W135. X,Y,Z) on the basis of polysaccharide capsular antigen
- Groups A, B & C are responsible for epidemics of meningitis
- Polysaccharides of groups A,C,W135 are immunogenic – used as vaccines
- Group B is not immunogenic.

DIAGNOSIS:

1.SPECIMEN: Blood, nasopharyngeal swabs, CSF, joints fluids.

2.Smears: Reveal gram-negative cocci in pairs which are coffee bean shaped.

3.Culture: Inoculate specimen on Chocolate agar plates; or Thayer-Martin Agar and incubate at 36 ^{o}C under 5-10% CO_2 tension.

4. Oxidase test: same as *N. gonorrhea*

Because meningococci may autolyse rapidly, the fluid must be examined fresh because in CSF, when out of the body, meningococci are quickly lyses.

TREATMENT

- Penicillin is drug of choice :
- penetrates well in inflammed meninges
- In penicillin hypersensitivity

Ceftriaxone

Effective against other two pathogens also i.e *H. influenzae* & **S. pneumoniae**

- At the end of therapy with penicillin (in adults): Give ciprofloxacin to eradicate meningococci because penicillin does not eradicate from nasopharynx: otherwise patient will become a carrier

PREVENTION

1. Treatment of carriers

•Rifampicin / ciprofloxacin

•Penicillin does not eradicate carrier state

◦ Due to inadequate penetration of uninflammed nasopharyngeal mucosa

- Poor secretion in saliva

2. Chemoprophylaxis to close contacts
   - •Ciprofloxacin
3. Meningococcal vaccine
   - •Divalent (gp A & C) &
   - •Quadrivalent (A,C,Y, W135)
   - •Not available against group B (not immunogenic)

   Given to:
   - °Military camps and pilgrims
   - °During epidemic

DIFFERENCES BETWEEN GONOCOCCUS AND MENINGOCOCCUS

1. Gonococcus grow more slowly, forms smaller colonies.
2. Gonococcus produces acid in glucose only, while meningococcus produces acid in both glucose and maltose.
3. Gonococcus is less toxic to mice and guinea pigs than meningococcus.

SIMILARITIES BETWEEN GONOCOCCUS AND MENINGOCOCCUS

1. Both are strict parasite and causes diseases only for man
2. They may show little differences in resistance to injurious agents.
3. Their distribution in the inflammatory exudates is the same.
4. They grow on artificial media with a little differences.

BRANHAMELLA

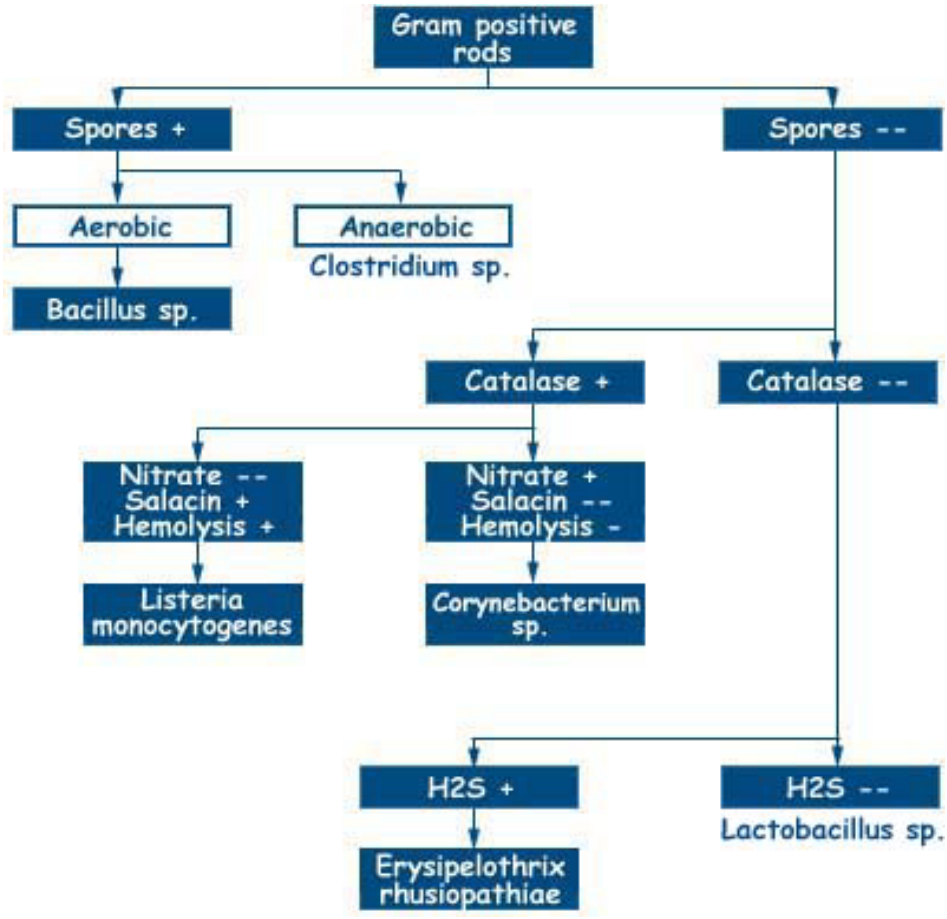
Branhamella catarrhalis (formerly called *Neisseria catarrhalis*). This is similar to *N. gonorrhea* and *N. meningitidis* morphologically and culturally. It is a part of the normal flora of the throat. It is normal inhabitant of mucus membrane especially the respiratory tract. Non-pathogenic characterized by its inability to ferment any sugar and by the ability to grow on routine laboratory media at room temperature. This was excluded from the genus Neisseria based on differences between the DNA structure.

Gram Positive Bacilli

MEDICALLY IMPORTANT
GRAM-POSITIVE BACILLI

AEROBES
Corynebacterium
Listeria
Bacillus
Gardnerella
Nocardia

ANAEROBES
Clostridia
Actinomyces

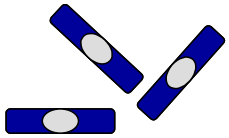


Gram Positive, Endospore-Forming Bacilli

THE GENUS BACILLUS

There are 48 species included in the genus bacillus with the following general characteristics:

1. Gram-positive bacilli
2. Spore former
3. Aerobic and facultative anaerobic



THE MOST IMPORTANT PATHOGENS:

1. Bacillus anthracis
2. Bacillus cereus

I. BACILLUS ANTHRACIS

Morphology:

1. Large gram positive bacilli
2. Non-motile
3. Found singly, in pairs or in long chains
4. Capsule could be demonstrated during growth in infected animals.
5. Spores are formed in culture, dead animal's tissue but not in the blood of infected animals.
6. Spores are oval and centrally located.

Survival in Soil

• Spores remain viable in soil for decades.
• In World War II in Scotland spores were exploded.
• Survived for >40 years and were eradicated in 1987
• Changing environmental conditions (temp. rain etc.) help in survival and multiplication.

Cultural Characteristics:

= Blood Agar and Nutrient Agar are commonly used to cultivate the bacilli. Plates are incubated aerobically at 37 ºC.
= **On blood agar plates**, colonies have irregular borders and are ***non-hemolytic***.
= **On nutrient agar:** They are described as "***Medusa head***" or ***"Comet tail".***

Specimen Collection and Laboratory Diagnosis:

CAUTION: Laboratory safety is very important when working with any materials suspected of containing Bacillus anthracis.

Samples are collected depending on the site affected:

1. Swab samples from cutaneous lesions and blood cultures.
2. Sputum and blood for pulmonary anthrax
3. Gastric aspirate, feces and blood for enteric anthrax.

= **Gram stained smears:** Made from clinical samples, show large gram positive bacilli in long chains "Bamboo-like appearance".
= **Giemsa stained smears:** Purple bacilli with red capsule.
= **Culture** See Cultural characteristics above.
=**Animal inoculation test:** Experimental animals are injected intraperitoneally by a suspension of the test organism "Suspected *B. anthracis* culture". The animal dies in 48-96 hours due to respiratory failure. Large number of typical bacilli can be found in the blood and tissue of spleen of the infected animal.
= Biochemical Identification:
1. Carbohydrate fermentation test:
2. Gelatine liqefaction test: Negative after 7 days. Growth has a characteristic appearance of an inverted pine tree.
3. Nitrate reduction test: Positive
4. Starch hydrolysis test: Positive
5. Voges-Proskauer test: Positive
6. Sensitivity to penicillin. Sensitive
7. Lysis by gamma phages: Positive. This test accurately differentiate *B.anthracis* from other bacilllus species.

Table 1. Shows some distinguishing characteristics of *B. anthracis*

Feature	***B. anthracis***	***B. cereus* and other *Bacillus spp.***
Hemolysis on sheep blood agar	Negative	Positive
Motility	Negative	Usually positive
Salicin Fermentation	Negative	Positive
Growth on PEA	Negative	Positive

PEA: PhenylEthyl Alcohol + Brain Heart Infusion Agar.

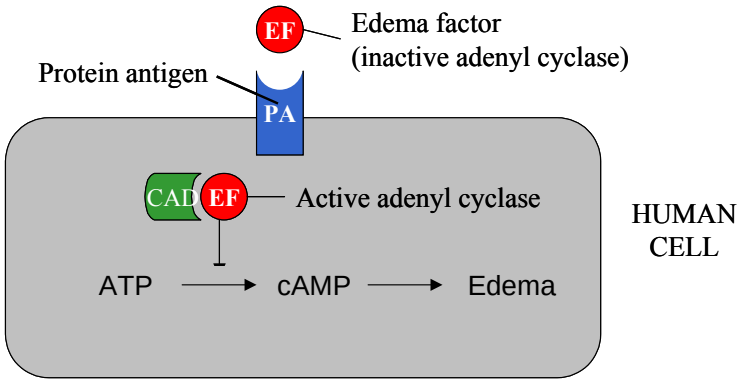
PATHOLOGY;

There are different clinical forms of anthrax:
1. CUTANEOUS ANTHRAX: 95-98% of anthrax cases are of this type. Infection occur through wounds, burns, which may progress to toxaemia and septicemia. The site of entry often produces a painless blister referred to as ***Malignant pustule***.
2. ENTERIC "INTESTINAL" ANTHRAX: Caused by the ingestion of infected meat. This form of the disease is severe and fatal.
3. PULMONARY ANTHRAX: Caused by the inhalation of large number of *B. anthracis* spores. It is usually fetal. This clinical form is commonly known as "***wool sorter disease".***

ANTIGENIC STRUCTURE AND PATHOGENIC DETERMINANTS:

1. The Capsular Polypeptide: Composed of poly peptide of a high molecular weight consisting of D-glutamic acid.
2. Polysaccharide Somatic Antigen: Composed of N-acetulglucoseamine and D-galactose
3. Complex Protein Toxin: This toxin appear to be responsible for signs and symptoms characteristic of anthrax. Accumulation of the toxin in tissue and its effect on the central nervous system results in death by respiratory failure and anoxia.

Pathogenesis of anthrax toxin



- Protein antigen (PA) is inserted into cell membrane of host cell
- Edema factor (EF)-inacive adenyl cyclase binds to PA
- PA facilitates transfer of EF into cytoplasm
- EF interacts with calmodulin (CAD) to become active adenyl cyclase

TREATMENT:

Penicillin is the drug of choice. For penicillin-sensitive patients, tetracycline, erythromycin, chloramphenicol and streptomycin may be given as alternative drugs.

BACILLUS CEREUS

- Gram-positive spore forming bacilli
- Produce β-hemolysis on blood agar

Pathogenesis & clinical features

° Spores are found on most raw foods like rice
° Spores are heat-resistant & survive rapid frying
° Produce enterotoxin – ingested → food poisoning.
° Short IP – 4-6 hours – similar to Staphylococcal food poisoning (vomiting & diarrhoea)

TREATMENT

- Symptomatic -fluid replacement
- Penicillin

CLOSTRIDIA

GENERAL CHARACTERISTICS:

1. Anaerobic gram-positive bacilli
2. Spore formers
3. They decompose proteins
4. Exotoxin producers
5. Natural habitat; Soil or the intestinal tract of animals and man.
6. Some motile, others are non-motile.
7. Catalase negative

SPECIES OF MEDICAL IMPORTANCE:

1. *Clostridium tetani*
2. *Cl. perfringens*
3. *Cl. botulinum*
4. *Cl. septicum*
5. *Cl. dificilli*
6. *Cl. novyi*

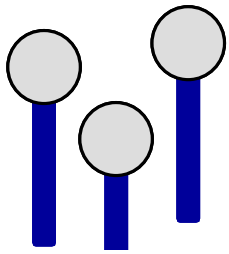
I. Clostridium tetani

Disease: Tetanus or "lock jaw"
Cl. tetani is not an invasive organism but rather remains strictly localized in the area of the affected tissue into which the spores has been introduced.

The disease is transmitted to man through infected wounds. Spores enter into wounds with soil or material contaminated with soil. The infection is localized in the area of entry. Growth and toxin production is aided by the followings:

1. Necrotic tissue
2. Calcium salts
3. Associated pyogenic infection.

These conditions aids in providing anaerobic conditions. The toxin "tetanospasmin" is heat-labile protein, inactivated by heating for 5 minutes at 65 ºC. The toxin may reach the central nervous system. It blocks the release of inhibitory mediators of the motor neurons. This results in extreme hyper-reflexia and violent spasm of skeletal muscles in response to any stimuli. This toxin is inactivated by the proteolytic enzymes of the digestive system. 1 mg pure toxin has 6 million lethal mice doses.

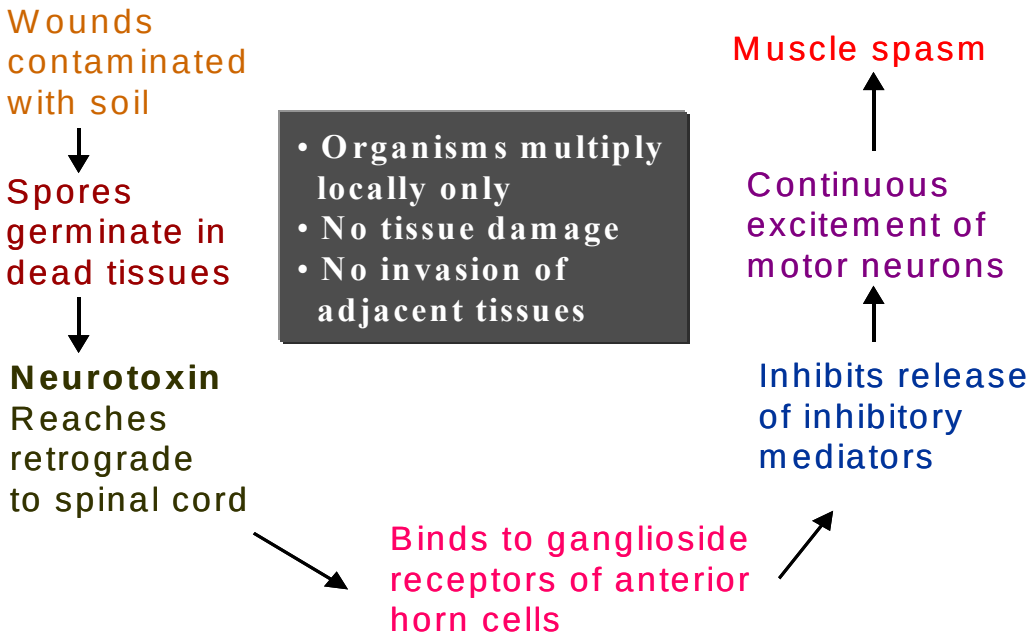


Morphology:
1. Gram-positive anaerobic
2. Motile with peritrichous flagella
3. Terminal rounded spores (Drumstick appearance).

Media of Choice:
Any medium with thioglycollate under anaerobic condition most especially Cooked Meat Media.

Pathogenesis of Tetanus

Occurs in traumatic open wounds contaminated with soil



II. Clostridium perfringens

Disease: Gas gangrene

It causes a variety of infection to man:

1. Wound infection
2. Gas gangrene
3. Necrotizing jejunitis
4. Food poisoning
5. Biliary tract infection
6. Meningitis

Four types of toxins are produced:

1. Alpha = It is a phospholipase C which hydrolysis lecithin. Disrupts cell membranes of RBCs, WBCs & muscle cells

2. Beta = Is necrotic for the intestinal mucosa and lethal to the CNS.

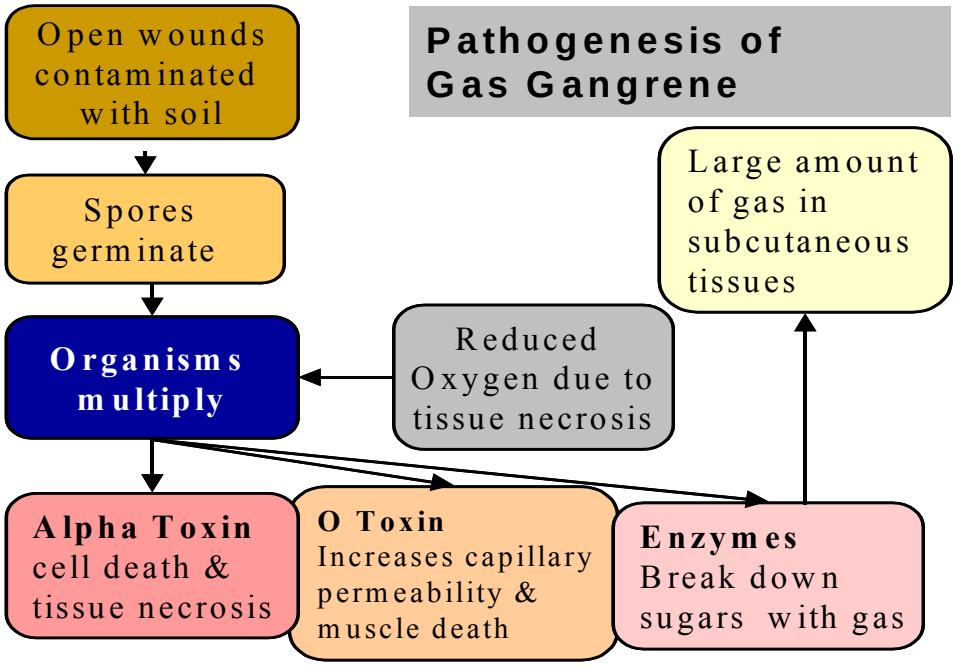
3. Epsilon = Inactive which is converted to the active form by trypsin in small intestine.

4. Iota = Similar to epsilon but differ in structure.

DISEASES By *C. PERFRINGENS*

1. Gas Gangrene (Myonecrosis)

Occurs in traumatic open wounds contaminated with soil



Morphological Characteristics:

= Spores are usually oval and central or subterminal.
= Not strictly anaerobe and can survive exposure to oxygen for short periods of time and sometimes referred to as aerotolerant anaerobe.
= Non-motile
= They form capsule in infected patients and animals.

Cultural Characteristics:

On blood agar plates: Colonies are round, domed and grayish white, surrounded by a zone of hemolysis.

III. Clostridium botulinum

BOTULISM: It is a disease caused by the ingestion of food containing the neurotoxin produced by *C. botulinum*. The earliest symptoms usually are an acute digestive disturbances followed by nausea and vomiting and possibly diarrhea, together with fatigue, dizziness and headache. Later there is constipation. Double vision may be evident early and difficulty in swallowing and speaking may be noted.

Patient may complain of dryness of the mouth and constriction of the throat.
Involuntary muscles become paralyzed, paralysis spread to the respiratory system and heart, the death usually results from respiratory failure.

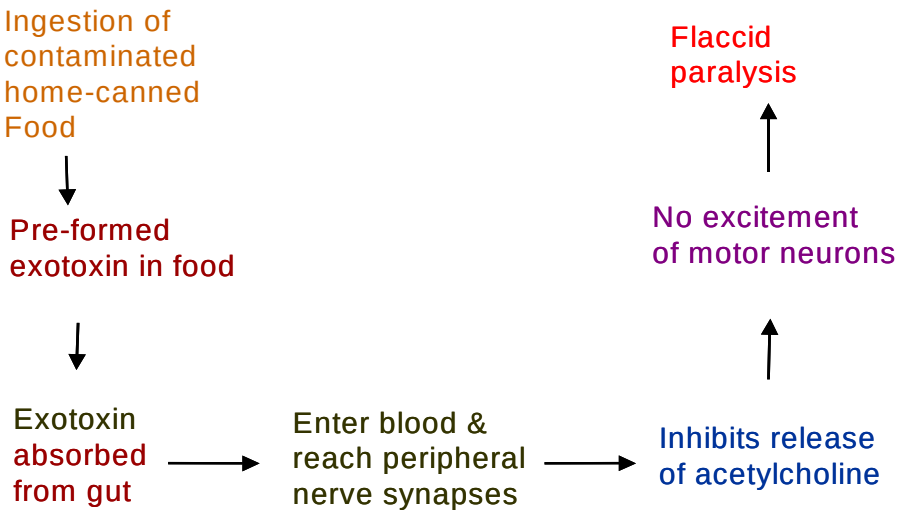
INFANT BOTULISM
Organisms introduced with dietary supplement
Organisms multiply in colon with absorption of small amount of toxin
Infant suffers from constipation & feeding problems
One of the causes of sudden infant death

Toxine Classification: 7 types has been identified (A-G). Only A, B, E, and F affects human.
1. Type A: Most common in the western parts of US. More toxic than B
2. Type B: Less toxic most frequent in occurrence
3. Type E: Obtained from fish and fish products
4. Type F: Similar to A and B. Has been isolated in Denmark.

NB: ***Botulinum toxin is the most powerful toxin known to man. 1 mg pure toxin has a 20 million lethal mice doses.***

Pathogenesis of botulism

Occurs due to consumption of contaminated home-canned food



Morphological Characteristics:
= Spores are oval, and subterminal
= Motile with peritrichous flagella

IV. Clostridium septicum

This organism sometimes causes rapidly progressive cases of Clostridial myonecrosis associated with severe wounds. Apparently, *C. septicum*, occasionally escapes from wounds and is carried by the blood stream to other parts of the body. Generalized *C. septicum* infection may occur in leukemia patients or by the administration of antimetabolite or other immunosuppressive drugs.

V. Clostridium difficile

Part of the normal flora of the human gut. It is the major cause of Pseudomembranous colitis that sometimes follows the administration of broad spectrum antibiotics for prolonged periods. Only when the other anaerobes in the intestine are killed or inhibited by certain antimicrobials, does *C. difficile* grow in sufficient numbers, and produce sufficiently great amount of toxin to cause colitis.

V. Clostridium novyi (A & B)

This organism is rarely associated with human infection other than **clostridial myonecrosis.** Type A strains are much more common than type B. Type B strain, almost unknown in human infections. They are primarily pathogens of sheep.

ISOLATION OF CLOSTRIDIA

1. From Clinical Sample that are Usually Sterile (e.g., Blood)
Inject the specimen in Blood Culture bottles with CO_2 and incubate anaerobically in an anaerobic jar. Subcultures onto freshly prepared Blood Agar are made and incubated anaerobically.

2. From Clinical Specimens that Contain a Mixture of Organisms (e.g., Abscess):

A. Heat Treatment: Specimen is inoculated to a tube of chopped-meat medium. Heat at 80 ^{o}C for 10 minutes before incubating anaerobically. Incubate overnight and subculture to Blood Agar and Egg-Yolk Agar.

B. Alcohol Treatment: 1 ml of the specimen is placed in a sterile screw-capped bottle or tube, add an equal amount of 50% ethanol for one hour. Pick up some of the treated materials, and inoculate egg yolk agar and blood agar and incubate anaerobically. A good selective media is **Neomycin-Egg-Yolk Agar.**

NB: Some Clostridium species may swarm. To prevent swarming, the amount of gar is increased to 4-6%.

NB: When growth is evident inoculate the following differential media for identification:

1. Egg-Yolk Agar	**For lecithinase & lipase production**
2. Chopped Meat Broth	**Digestion; indole production**
3. Milk Agar	**Digestion**
4. Gelatine agar	**Liquefaction**
5. Glucose Broth	**Fermentation**
6. Maltose Broth	**Fermentation**
7. Lactose broth	**Fermentation**
8. Sucrose broth	**Fermentation**
9. Salicin Broth	**Fermentation**
10. Mannitol Broth	**Fermentation**

IDENTIFICATION OF CLOSTRIDIA:

= To differentiate between Clostridia and Bacillus especially *B. cereus* which grow well under anaerobic conditions, catalase test is performed which is negative for Clostridia and positive for Bacillus.
= *C. perfringens* produces lecithinase (phospholipase C)
= Other species produces lipase that break down fatty acids

Table 1. Distinguishing tests for the different Clostridia species

Species	Milk	Lecithin	Lipase	Gel	Ind	glu	mal	suc	sal	man
1.***C. tetani***	-	-	-	+	+	-	-	-	-	-
2.***C. perfringens***	-	+	-	+	-	+	+	+	V	-
3. ***C. botulinum***	D	-	+	+	-	+	+	-	V	-
4. ***C. septicum***	-	-	-	+	-	+	+	-	V	-
5. ***C. difficile***	-	-	-	V	-	+	-	-	V	+
6. ***C. novyi* (A)**	-	+	+	+	-	+	+	-	-	-

V= Variable reaction
D = Digestion of milk
C. perfringens produces double zone of hemolysis.

NB: Identification of toxigenic strains is done in a similar way to that of diphtheria by toxin neutralization in Vivo.
NB: If *C. perfringens* is to be specifically isolated, incubate the plates at 44-46 ^{o}C.

TREATMENT;
1.TETANUS: Since treatment of tetanus are not satisfactory, prevention is all-important. Prevention of tetanus depends upon:

1. ***Active immunization with toxoids***
2. ***Proper care of wounds, contaminated with soil***
3. ***Prophylactic use of antitoxin***
4. ***Administration of penicillin***.

2. BOTULISIM: Potent antitoxin to 3 types of botulinus toxins have been prepared. Trivalent (A,B,E) antitoxin is administered intravenously as early as possible to neutralize the effect of the toxin.

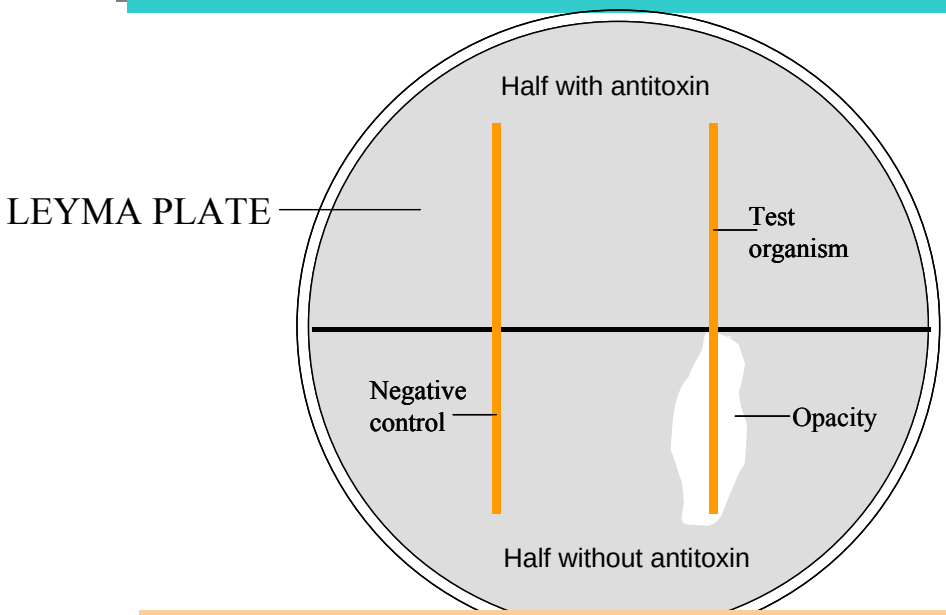
3. GAS GANGRENE: Treatment of the disease is done by :

1. ***Surgical measures to remove all devitalized tissues.***
2. ***Administration of penicillin***
3. ***Administration of antitoxin.***

4. Infection due to *C. septicum* and *C. novyi* : Similar to gas gangrene

5. Pseudomembraneous colitis: Administration of oral vancomycin is effective in reducing the number of this organism in the human intestine.

Nagler's Reaction for *C. perfringens*



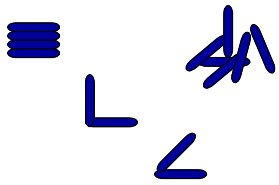
- Divide LEYMA plate into two halves.
- With a sterile swab cover one half with *C. perfringens* antitoxin & dry it
- Inoculate the test organism at right angles to center line.
- Similarly inoculate a negative control
- Incubate anaerobically.
- Opacity in around inoculum of test organism in half without antitoxin and
- No opacity in half with antitoxin

CORYNEBACTERIUM

OFFICIAL SPECIES:
1. *Corynebacterium diphtheria*
2. *Corynebacterium ulcerans*
3. *Corynebacterium ovis (pseudotuberculosis)*

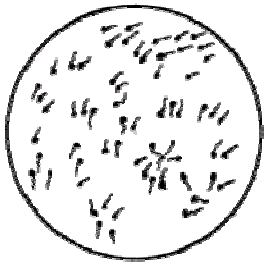
I. Corynebacterium diphtheria

Clinical manifestation:
=Local lesions in the nose and throat
=Primary infection may affect ear, conjuctiva, umbilicus and vagina.
=The classic form of the disease is characterized by a local pseudomembraneous lesion covering the tonsils, pharynx or in the nose. The pseudomembrane can extend into trachea and completely obstruct the air passage, causing the patient to die of suffocation.
=Diphtheria toxin produced in the local lesion which is absorbed systematically can damage such distant organs and tissues such as heart, liver, kidneys and central nervous system. Death is usually results from heart failure.



General Characteristics:
= Gram-positive bacilli, Pleomorphic (Curved or straight)
= Non-motile, non-acid fast and aerobic.
Outstanding Characteristics:
= Club-shaped forms = Swelled end
= Chinese characters "Cell arrangement L or V shapes are also observed
= Ability to grow on selective media containing potassium tellurite.

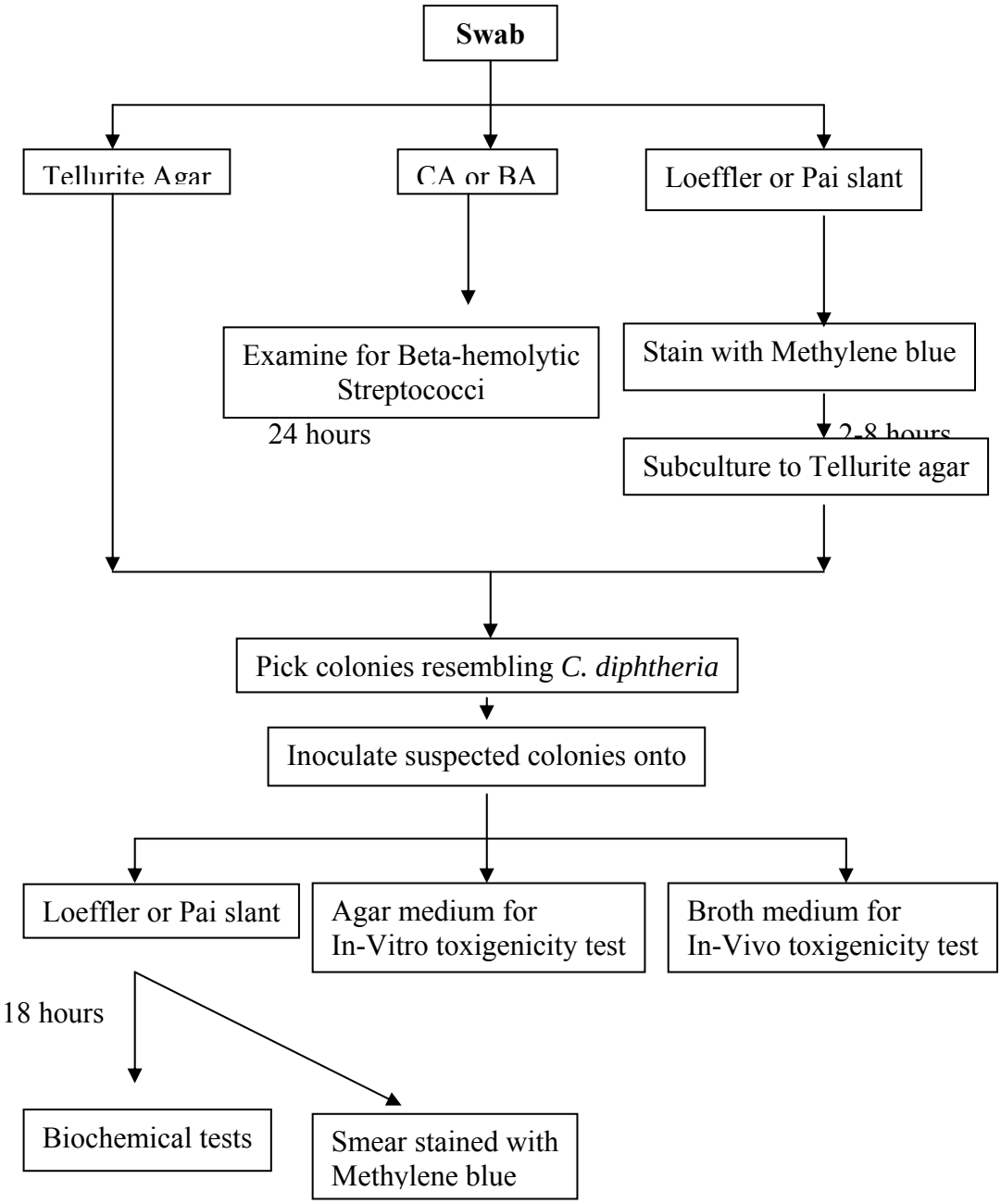
Specimen Collection:
= Throat and nasopharengeal = When diagnosis is confirmed, patient contacts must be subjected to the same procedures. Moisten swabs with NSS and collect in the usual manner.



Media:
1. Pai medium
2. Tinsdale medium
3. Loeffler coagulated serum medium
4. Chocolate agar with potassium tellurite.

Pai medium and Loeffler medium provides good growth of *C.diphtheria* and stained smears prepared from both media may show the characteristic Chinese letters blue bacillus.
Colony Morphology:
Gray to black with irregular edges on Loeffler medium. Based on colony morphology Corynebacteria are divided into 3 groups:
1. Gravis: Large, flat, gray to black, non-hemolytic
2. Mitis: Smaller, more convex, darker with dull surface
3. Intermedius: The smallest of all
Note: Gravis strain hydrolysis starch and glycogen while mitis and intermedius do not.

Isolation and Identification:



Biochemical tests:

The colonial morphology of *C. diphtheria* on Tinsdale medium is the most important characteristics in differentiating it from other species of corynebacteria.

=Urease Negative
=Nitrate Positive
=Gelatin liquefaction: Negative
Fermentation reactions of various carbohydrates.

For accurate differential characteristics, refer to the following table.

Table: Differentiation between major species of Corynebacteria

species	Tinsdale agar-halo	catalase	urea	nitrate	gelatin	glucose	sucrose
C. diphtheria	+	+	-	+	-	+	-
C. ulcerans	+	+	+	-	+	+	V
C .ovis	-	+	+	+	-	+	V

Toxigenicity Tests:

1. In-Vivo toxigenicity test:

1. From a pure culture of an isolate, inoculate a 10-ml tube of BHIB, incubate at 37^0C for 48 hours.
2. Inject one guinea pig with diphtheria antitoxin to serve as a control.
3. After 2 hours, inject 5 ml of the broth into both the control and test animals.
4.Observe after 48 hours.

Results and interpretation

= If the test animal dies and the control survives= It is *C. diphtheria*

= If both the test and control show illness or die= It is another organism.

 **Test**	 **Control**
No injection with diphtheria antitoxin	**Injected with diphtheria antitoxin**

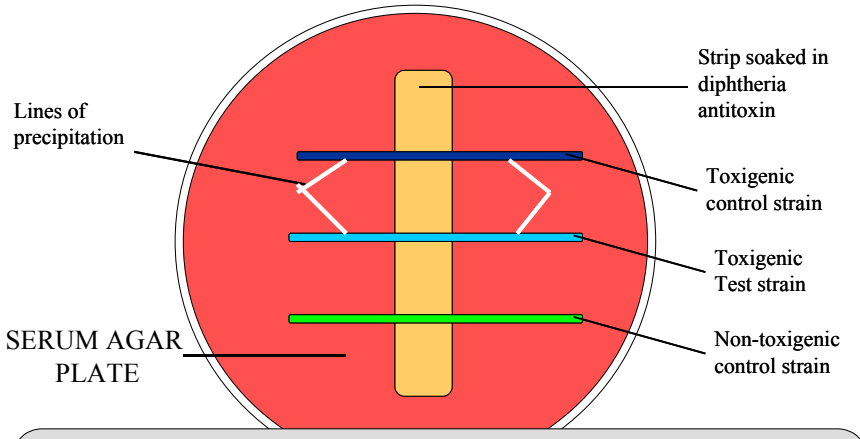
2. In Vitro toxigenicity test:

Principle: This test is based on a precipitin reaction between diphtheria toxin produced by the test culture and diphtheria antitoxin in the medium. The reaction produces insoluble products that precipitate.

Procedures:

1. Paper strip is saturated with diphtheria antitoxin.
2. The medium contained in a petri-dish is streaked with the test organism or organisms if more than one strain are to be tested.
3. Place the paper strip perpendicular to the organism streaks.
4. Incubate the plates for 24 hours at 37 ^{o}C.
5. Examine the line of precipitation that extend out from the line of bacterial growth.
6. The presence of line of precipitation is considered positive and it confirms that the test organism as a toxigenic.

ELEK'S TOXIGENICITY TEST FOR *C. DIPHTHERIAE*



- Soak a strip of sterile filter paper in diphtheria antitoxin (750 units / ml) – lay it in a petri dish.
- Pour 20 ml of sterile rabbit serum agar onto the strip – let the medium cool
- Inoculate the test and +ve & -ve controls organisms as shown in figure
- Incubate overnight at 37°C and look for lines of precipitation

Skin test= Schick test= To determine whether The patient is susceptible to diphtheria infection or not by detecting the presence or absence of antibodies.

Procedures:

1. 0.1 ml of toxin is injected intradermally in one arm.
2. 0.1 ml of heated (inactivated) toxin as a control is injected in the other arm.
3. Read after 24-48 hours.

Positive: **(Susceptible)**Redness and swelling that increases for several days and then fades, leaving brownish pigmented area. The control site shows no reaction.

Negative:(Not susceptible) Neither injection site shows any reaction).

Treatment:

1. Antibiotic therapy: Penicillin or erythromycin=To eradicate the organism
2. Anti-toxin: To neutralize the effect of diphtheria toxin.

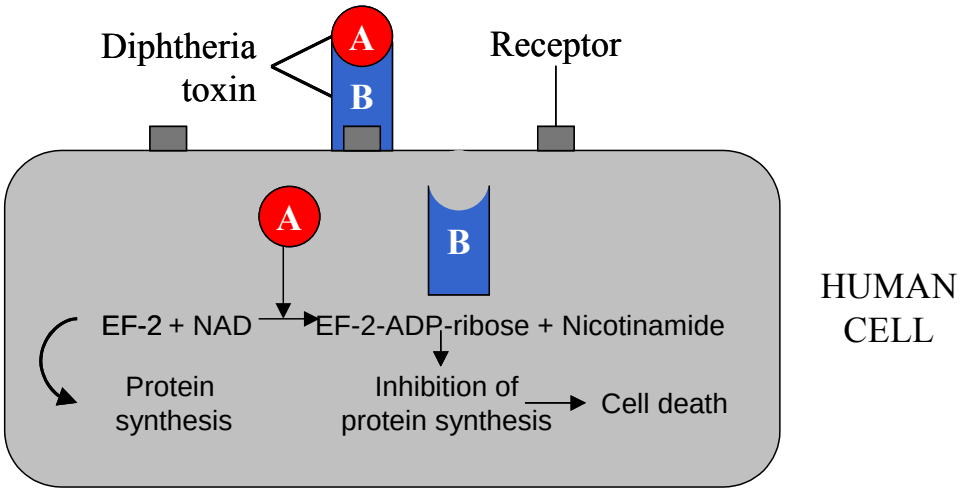
Control: By immunization with inactivated or attenuated toxin of toxigenic *C. diphtheria*.

Diphtheria toxin:

1. Structure: It is a heat-labile polypeptide that can be lethal in a dose of 0.1 ug/kg. If disulfide bond are broken, the molecule can give two fragments. (A & B). Fragment B has no independent action but it is required for the transport of fragment A.

2. Mechanism of Action: Fragment A inhibits polypeptide chain elongation by inactivating the elongation factor 2 "EF2". This factor is required for translocation of polypeptidyl-transfere RNA from the acceptor to the donor site on the eukaryotic ribosome. Thus preventing protein synthesis leading to cell death.

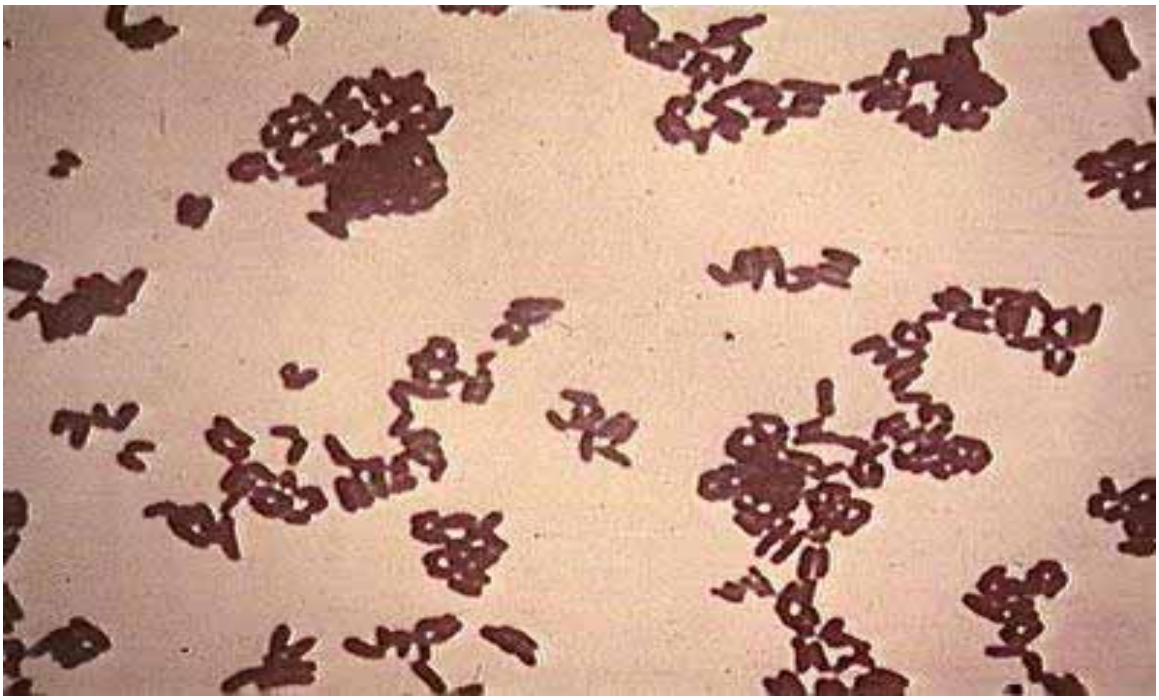
Pathogenesis of diphtheria toxin



ADP ribosyulation of EF-2 (elongation factor-2)

- Subunit B: Binding subunit - help attachment to cell receptors
- Subunit A: Active subunit- cleaves nicotinamide from NAD and transfers the remaining ADP-ribose to EF-2 (ADP-robosylation)
- Inactivates EF-2 and shuts of protein synthesis - cell death

Note: *C. ulcerans* and *C. ovis* also produce diphtheria toxin but to a lesser extent and could be differentiated either morphologically or biochemically.



Erysipelothrix insidiosa
E. rhusiopathiae

Disease: Erysipleoid (red skin) = it has three distinct clinical enteities:
1. Erysipeloid : Localized cutaneous infection usually on the finger and hands.
2. Generalized cutaneous infection.
3. Septicemia

Organism:
- Gram-positive rods
- Non-motile
- Non-spore forming
- Facultative anaerobe

Specimen and media:
- Usually skin biopsy from the advancing borders of the lesion.
- Blood specimen if septicemia is suspected.
- Culture media: **1% glucose broth** and **blood agar**

NB: Make gram staining for the organism from the clinical specimen and the growth on blood agar.

Biochemical tests used in the identification:
- Test tube brush appearance in gelatine stabs
- H_2S production
- Acidification of slant and butt in TSIA with no gas production.
- To differentiate this organism from *Listeria monocytogenes*, perform the catalase test. *Listeria monocytogenes* is catalase Positive.

Treatment:
- Penicillin is the drug of choice

Listeria monocytogenes

Disease: Listerosis = it has four forms
1. Non-specific flu-like illness during pregnancy or puerperal sepsis
2. Neonatal sepsis and later meningitis
3. Sepsis or meningitis in immunocompromised patients.
4. Food poisoning.

Organism:
- Gram-positive rods (Shorter & Thinner).
- Non-spore forming
- Facultative anaerobe
- Beta hemolytic on Blood agar.

Biochemical Characteristics:
- Motile (Umbrella-type growth in semi-solid media)
- Motile (Tumbling motility as seen by the hanging drop technique)
- Catalase (+)
- V.P (+)

Specimen and Culture media:
- Blood, cerebrospinal fluid (CSF) and genital tract secretion.
- Media : BHIA + 5% sheep blood and BHIB.
- Selective Listeria Agar SLA = Brownish to black discoloration is usually seen around the colonies of *Listeria monocytogenes.*

Identification:
1. The demonstration of Gram-positive bacilli from stained smears (CSF and other fluids or food samples).
2. Culture and Isolation: On Blood Agar or Selective Listeria Agar
3. Biochemical tests: Catalase test, V.P, and motility test
4. Serological test: Agglutination with specific sera.

Treatment: Combined therapy of penicillin plus aminoglycosides. Tetracycline also may be used as a second line if the patient is sensitive to penicilllin or aminoglycosides.

MYCOBACTERIA

SPECIES OF MEDICAL IMPORTANCE:

1. Mycobacterium tuberculosis complex

- *M. tuberculosis*
- *M. bovis*
- *M. africanum*

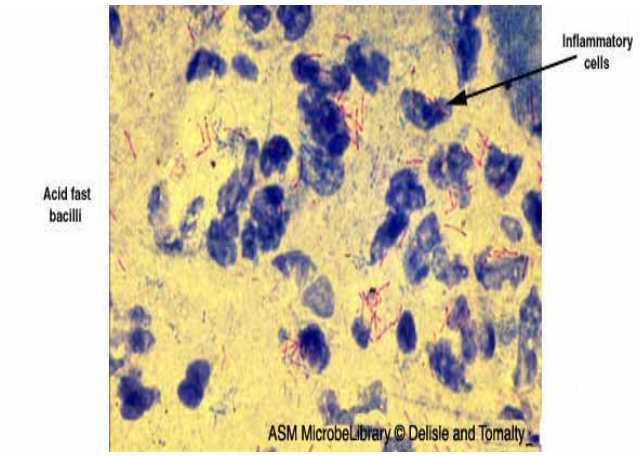
2. Non-tuberculosis mycobacteria (NTM)

a) Atypical mycobacteria

- *M. avium-intracellular*
- *M. marinum*
- *M. scrofulaceum*

b) Non-cultivable

- *M. leprae*



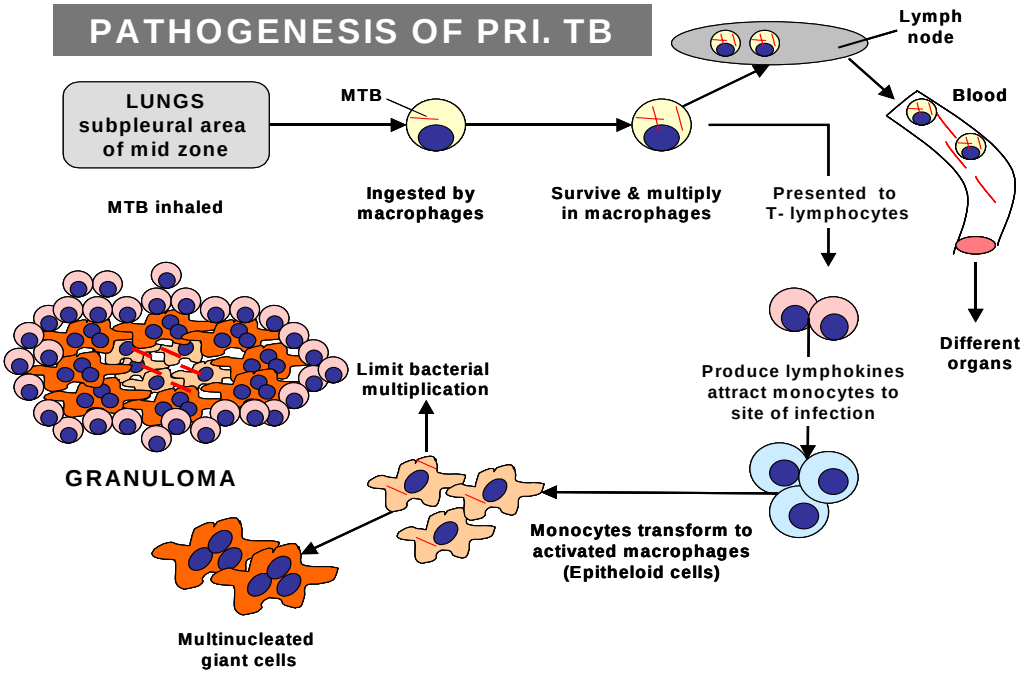
General Characteristics of Mycobacteria:

1. Acid fast straight or slightly curved rods.
2. Non- motile
3. Non-spore forming
4. Aerobic (growth is enhanced by the presence of 5-10% CO_2
5. Slow growing bacteria (Average doubling time is 12 hours), fastidious in nutrition.
6. Resist staining with basic dyes as a result of their high-cell wall lipid contents.

I. Tuberculosis
Mycobacterium tuberculosis

Clinical Manifestation:

Organisms is droplets of 1-5 um are inhaled and reaches alveoli. The disease results from establishment and proliferation of virulent organisms and interaction with the host. The production and development of lesions and their healing or progression are determined chiefly by the number of mycobacteria and their multiplication and the resistance and hypersensitivity of the host. Two types of lesion are produced as a result of *M. tuberculosis* infection:



1. Exudative type: This consist of an acute inflammatory reaction, with edema fluid, polymorphonuclear cells, and later, monocytes around the tubercle bacilli. This type is particularly seen in lung tissues, where it resembles bacterial pneumonia.
2. Productive type: When fully developed, this lesion, a chronic granuloma, consist of three zones:

A. A central area of large multinucleated giant cells containing *M. tuberculosis*.
B. A mid-zone of pale epithelial cells
C. A peripheral zone of fibroblast, lymphocytes and monocytes.

This lesion is called a tubercle. Septicemia usually occur early in the course of infection and meningitis is not uncommon.

Specimen:
Fresh sputum, gastric aspirate or washing, urine, pleural fluid, cerebrospinal fluid, joint fluid, biopsy materials.

Stained smears:
Sputum or sediments from other body fluids is stained for acid fast bacilli by Ziel-Neelsen technique.

Culture: Sputum must undergo concentration procedures prior culturing:
Procedures:
1. Mix an equal volume of sputum with 4% sodium hydroxide containing 0.004% phenol red in 100 ml tube containing glass beads. Homogenize by shaking for few minutes.
2. Centrifuge at 3000 rpm for 20-30 minutes.
3. Decant the supernatant fluid into a disinfectant, leaving little fluid to resuspend the sediments. Add one drop of phenol red indicator.
4. Using sterile pipette, add 2N HCl drop by drop to a definite yellow end point.
5. Inoculate the sediments to egg media. Two Lowenstein-Jensen slopes.
6. Prepare a smear from sedimented materials and stain with Acid-Fast staining procedures.

NB: Incubation of the un inoculated media is continued for up to 8 weeks.
Increased CO_2 tension enhances growth
NB: Colony morphology: Bread-crumbs appearance.
Lowenstein Jensen Medium – growth in 4-6 weeks
Middlebrook Agar (7H11) – growth 2-3 weeks

Biochemical Identification:

1. Niacin test: Only *M. tuberculosis* and *M. simiae* are positive for this test
PROCEDURES:
1. Add one ml of sterile distilled water to a 3-4 weeks old culture on L.J medium and hold the tube so that the water covers the medium around the colonies.
2. After 15 minutes, remove 0.5 ml of the liquid containing extracted niacin, and transfer it to a clean, screw-capped test tube.
3. Add 0.5 ml of **4% aniline solution** and 0.5 ml of **10% cyanogen bromide**.
4. If yellow color appears immediately, niacin is present.

NB: To differentiate *M. tuberculosis* from *M. simiae*. *M.simiae* produces pigment while *M. tuberculosis* does not.

Other Biochemical Tests:

1. Nitrate reduction test
2. Urease test
3. Tellurite reduction test
4. Sodium chloride tolerance test
5. Pigment production test
6. Catalase test
7. Arylsulfate test
8. Growth on MacConkey agar

SEROLOGICAL TEST:

1. Skin test: Tuberculin test: An intradermal injection composed of one of these extracts:

1. Old tuberculin: Crude extract from the filtrate of *M. tuberculosis* broth culture. Also known as Tuberculin-Koch.

2. Purified Protein Derivatives: (PPD): Purified extract.

TUBERCULIN (MONTOUX) TEST

Purified Protein Derivative (PPD) from tubercle bacilli (standardized) in Tuberculin Units - TU) is injected intradermaly

DOSE

5 TU is usual dose – if test negative; increase dose to 250 TU

POSITIVE TEST

The test is read after 48 – 72 hours

Induration of ≥ 10 mm with erythema

Induration of 5-9 mm – low level sensitization with tubercle bacilli or cross reacting mycobacteria.

In AIDS patients: 5 mm induration – positive test.

INTERPRETATION OF TUBERCULIN TEST

Positive Test

- Active disease
- Person infected by *M. tuberculosis* at sometime in life
- Person infected with strongly cross reacting other mycobacteria
- Previous vaccination with BCG
- Child < 5 years if not vaccinated: active disease.

Negative Test

- No induration or < 5 mm
- In healthy individual – not infected with MTB
- Pre-hypersensitivity stage of primary infection

False negative test

- Early TB (test becomes positive after 4-6 weeks of infection)
- Miliary TB
- Immunosuppression (AIDS)
- Steroid therapy

Treatment:
Because *M. tuberculosis* infections are hard to treat and only few antibiotics has activity against it, and because of the fear of emerging of resistant strains, tuberculosis is treated with 2 or 3 antibiotics given as combined therapy.
Active antibiotics against *M. tuberculosis* include:
1. Isonizide (INH)
2. Rifamycin
3. Streptomycin
4. Ethambutol
5. Para-aminosalicylic acid PAS
6. Cycloserine
7. Pyrazinamide
8. Viomycin

Treatment for complete recovery may take as long as one year. The shortest term may be of 6 months with the administration of combined drugs (Isonizid, rifamycin, streptomycin).

ATYPICAL MYCOBACTERIA
M. avium – interacellulare complex

Worldwide distribution
Source – natural water
Diseases
TB in birds
Cervical lymphadenitis in children
Disseminated TB in AIDS patients

DIFFERENCES OF ATYPICAL FROM TYPICAL MYCOBACTERIA
Colonial morphology
Niacin test – negative
Relatively more resistant to anti-TB drugs
Less acid fastness
Diseases less invasive

II. *Mycobacterium leprae*
Leprosy

General Characteristics:
= Typical acid fast bacilli, singly or in parallel bundles.
= Regularly found in smears or scrapings from skin or mucus membrane in lepromatous leprosy.
= The organism have not been grown on artificial media.

Clinical manifestation:
The lesions involve the cooler tissue of the body, skin, superficial nerves, nose, pharynx. The skin lesions may occur as pale, anesthetic 1-10 cm in diameter. Neurologic disturbances are manifested by nerve infiltration and thickening.

The disease is divided into two distinct types:

1. Lepromatous leprosy: The course is progressive, with nodular skin lesions; slow, symmetric nerve involvement, abundant acid-fast bacilli in the skin lesion; continuous bacteremia and negative skin test **(lepromin test)**

2. Tuberculoid leprosy: The course is non-progressive with macular skin lesions, severe asymmetric nerve involvement of sudden onset with few bacilli present in the lesion.

Diagnosis:
Scrapping with a scalpel blade from skin, nasal mucosa, or from a biopsy of ear lobe skin are smeared on a slide and stained by Zeil-Neelsen technique, and the demonstration of typical acid fast bacilli.

Treatment:
Combined therapy of dapsone, rifamycin and clofazimine

Francisella tularensis
Tularemia "Rabbit fever"

Characteristics of the Organism:

1. Gram-negative rods "Shows variable shapes ranging from coccobacilli, to cocci"
2. Strict aerobe
3. Requires enriched medium for growth "Cystine-Glucose-Blood Agar" **CGBA**
4. Non-motile
5. Remain viable for long periods in water, soil.....etc.
6. Highly infectious. Specimen and cultures must be handled with great care.

Clinical Manifestation:

Tularemia in human vary according to:

1. Route of infection
2. The infecting dose
3. The type of organism (A or B strain)

The disease may be transmitted through the skin and mucus membrane including the conjuctiva through the bite of infective ticks. Transmission may also occur through the respiratory route or gastrointestinal tract through ingesting contaminated meat of infected animals.

THE ULCEROGLANDULAR: The most common, with slowly healing ulcer at the site of entry and regional lymph nodes involvement is observed. This infection results from injury while handling infected animal carcasses or from the bite of a blood-sucking arthropod such as ticks and flies.

OCULOGLANDULAR: Can result from rubbing contaminated materials into the eye. Conjuctivitis occur and regional lymph node may be enlarged and suppurate.

THE PULMONARY OR PNEUMONIC: Can result from inhaling infectious aerosols or dust. Pneumonia and pleuritis are associated with severe tularemia.

TYPHOIDAL, GIT & OROPHARYNGEAL INFECTION: Results from the ingestion of contaminated food. Symptoms begin as flu-like associated with vomiting, headache, fever and prostration. Diarrhea usually accompany these symptoms.

Subspecies of *F. tularnsis*

1. *F. tularensis* A : More virulent than B. It is also referred to as tick-deerfly-sheep type and occurs naturally in North America.

Fatality rate among untreated patient is 5-7%.

2. *F. tularensis* B: It is referred to as the Beaver-Muskrat water type. It produces mild (Subclinical) infection in human. It have frequently been isolated from natural running water.

NB: To differentiate between the two, 1% glycerol is added to Cystin-glucose-heart agar without blood and enough bromothymol blue indicator.

TYPE A: Turns the medium yellow (acid)

TYPE B: Turns the medium dark blue (alkaline)

Collection of specimen:

1. Exudates from cutaneous or mucosal ulcers or lymph node aspirate.
2. Blood or sputum for pulmonary infection
3. Blood specimen for serologic for measuring antibody titer
4. Specimens (biopsy) from enlarged liver or spleen.,

Diagnostic procedures:

A. Microscopic appearance:

- Short gram-negative non-motile bacilli
- Morphologically may be similar to **Yersinia** species and shows bipolar reaction.

B. Cultural Characteristics

- On CGBA: colonies resemble a medium sized mercury droplets surrounded by a small zone of partial hemolysis. Colonies often requires 2-4 days at 37 ^{o}C to reach a diameter of 1 mm.

C. Inoculation of the specimen:

- Primary isolation from specimen may be difficult even though it may contain large numbers of *F. tularensis*.
- Contaminating organisms can easily overgrow cultures or produce large amounts of acids to prevent the growth of *F. tularensis* on the enriched media needed for isolation.
- Cystine glucose blood agar is the medium of choice. It is commercially available or can be prepared in the laboratory.

D. Biochemical Identification:

- Biochemical tests are of a little value in identifying the organism.

1. Tube agglutination test:

2. The slide agglutination method:

3. The precipitin test:

Can be used to measure antibody titer and demonstrate the presence of antigen. This test is useful for grossly contaminated, even decayed specimen such as liver and spleen of naturally infected, field-collected animal specimen,

4. Hemagglutination and complement fixation tests are of limited use.
5. Skin test have been used for prevalence of past infection in large population.

Treatment and antibiotic sensitivity:

Streptomycin, Chloramphenicol and tetracycline are used to eradicate this organism.

GENUS: HAEMOPHILUS

SPECIES:
1. *H. influenza* (Biotype B is the most important)
2. *H. parainfluenza*
3. *H. haemplyticus*
4. *H. ducreyi*
5. *H. aphrophilus*
6. *H. paraphrophilus*
7. *H. segnis*

General Characteristics:
1. Gram-negative small rods (pleomorphic and may show variable gram reaction and has poor absorbance of stain)
2. Facultative anaerobe
3. Require CO_2 for growth
4. Require growth factors such as factor X (hemin) and factor V (NAD)
5. May be capsulated or non-capsulated.
6. Most strains are indole positive.

H. influenza

Pathogenicity:
In human, *H. influenza* is the most common etiologic agent of acute bacterial meningitis in young children. *H. influenza* is the most important pathogen among the other species of the genus Haemophilus. Among the most common infections caused by this organism are:

1. It is the most common cause of meningitis in children
2. Cause epiglotitis
3. Septic arthritis
4. Bronchitis and pneumonia
5. Acute and chronic otitis media
6. Endocarditis and pericarditis.
7. May enter the blood stream of women during birth causing puerperal sepsis, the complication of which may threaten the mother life.
8. Eye infection (Conjuctivitis)

Normal Habitat:
It forms part of the normal flora of the upper respiratory tract of man.

Speciemn:
C.S.F, nasopharengeal specimen, blood, pus and sputum for culture. As a general precaustion, specimen suspected of containing *H.influenza* must not be refrigerated. For *H.ducreyi*, a genital specimen is required.

Microscopy:
Small, non-motile, gram-negative (may show gram variable reaction). Bacilli with long thread-like forms of *H. influenza* may be seen in CSF or older culture.

Culture:
=Aerobic growth is preferred over anaerobic.
=Chocolate Agar is a good medium which if prepared properly could provide both X and V factors. Colonies on CA are smooth, grayish and minute.
=Increased CO_2 tension is necessary for growth.

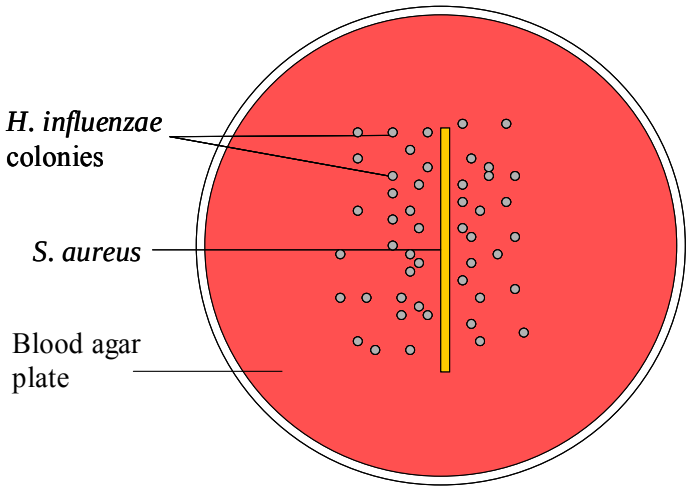
what are X and V factors ?
1. X factor: (HEMIN) It is used by the organism to produce essential respiratory enzymes such as cytochromes, catalase and peroxidase.
2. V factor: (NAD) (Nictonamide Adenine Dinucleotide) Used as electron carrier in the Oxidation-Reduction system.

Identification:
H. influenza is primarily characterized based on its requirement for X and V factors.

SATELLITISM TEST : to identify H.influenza
1. Mix a loopful of suspected Haemophilus growth in about 2 ml of sterile NSS or sterile peptone.
2. Using a sterile swab, inoculate the organism suspension on a plate of NA and BA.
3. Streak a pure culture of *S. aureus* across each of inoculated plates.
4. Incubate both plates in CO_2 enriched atmosphere at 35-37 ^{o}C.
5. After an overnight incubation, examine the culture for growth and satellite colonies.

Results and Interpretation:
H.influenza: Growth on BA as satellite but no growth on NA.
Other Haemophilus: Growth on BA as well as NA. This mean that the organism requires only factor V.
What is satellitism ? The colonies near the column of *S.aureus* are larger than those furthest from it.
NB: *H.influenza* require both factors.

SATELLITISM BY *H. INFLUENZAE*



Biochemical Identification: (seldom used)
Biochemical tests are used to differentiate species in the genus Haemophilus. The following tests are commonly employed:
1. Hemolysis on Blood agar
2. Indole test
3. Growth factors requirements: (factor X and V)
4. Urease test.

Table Growth requirements and hemolytic activity of Haemophilus species.

Haemophilus	Growth factors		Hemolysis on BA
	X	V	
1.H. influenza	+	+	-
2. H. parainfluenza	-	+	-
3. H. haemolyticus	+	+	+
4. H. aphrophilus	+	-	-
5. H. ducreyi	+	-	slight
6. H. segnis	-	+	-
7. H. paraphrophilus	-	+	-

Treatment:
Ampicillin if sensitive is the drug of choice.
= Chloramphenicol
= Tetracycline

HAEMOPHILUS DUCREYI

Gram-negative coccobacilli
Cultured on chocolate agar
Require only X factor
Causes Chancroid
A sexually transmitted disease with soft ulcers on external genitalia with local lymphadenitis (bubo)
Treatment:
Ceftriaxone or ciprofloxacin

GARDENERELLA VAGINALIS

Gram-negative coccobacilli
Formerly called Haemophilus vaginalis
Found in small numbers in vaginal secretions of 50% of normal women

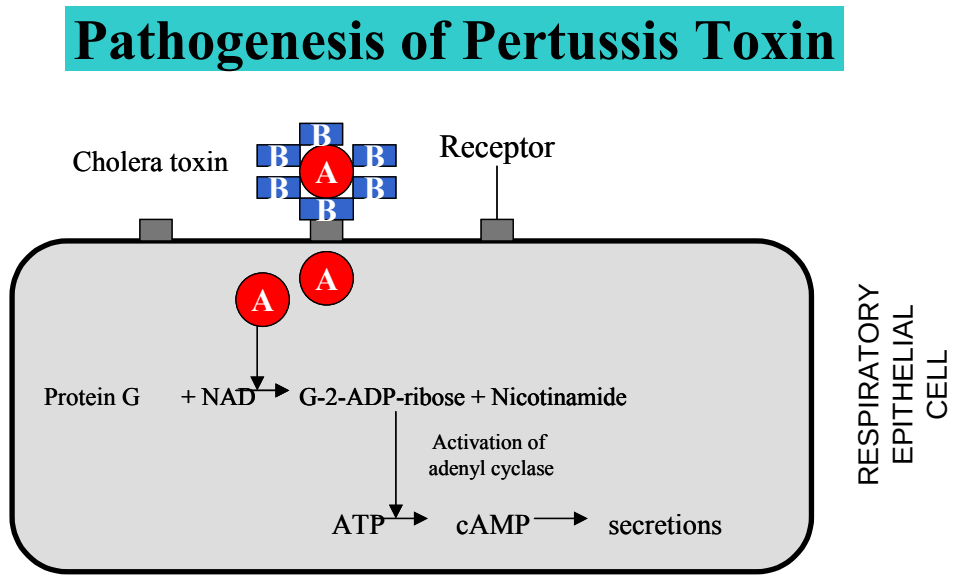
Disease: Bacterial Vaginosis
Malodourous vaginal discharge

Lab Diagnosis
Gram-smear of Vaginal discharge
Clue cells (epithelial cells covered with bacteria)

Bordetella pertussis

Disease:

Pertussis = Transmitted by the respiratory tract rout from early case and possibly via carriers. The organism adheres to and multiply rapidly on the surface of the epithelium in the trachea and bronchi and interfere with ciliary action . Disintegrating organisms liberate a toxin that irritate surface cells, giving rise to catarrhal symptoms. Most *B. pertussis* strains contains peptide that promotes marked lymophocytosis in the host. The toxin is heat stable.



ADP-ribosyulation of Protein G

- Subunits B facilitate entry of subunit A (Active subunit) into cell
- Subunit A cleaves nicotinamide from NAD and transfers the remaining ADP-ribose to protein G
- Activates adenyl cyclase to convert ATP to cAMP - secretions

Organism:

- Short, ovoid, gram-negative bacilli
- Aerobic
- Complex, enriched media is required for isolation (Bordet-Gengou)

Laboratory Diagnosis:

- Nasopharyngeal swabs or cough droplets, expelled onto "Cough plates" held in front of the mouth of the patient during a paroxysms.
- Incubate **(Bordet-gengou`s plates)** at 35 ^{o}C for 2-5 days.
- Typical colonies resemble a 1mm droplets of mercury surrounded by a zone of hemolysis.
- Colonies are confirmed with specific antiserum
- Direct immunofluorescent staining of smears made from nasopharyngeal swabs may give a rapid positive test.

Other Species Differentiation

FEATURE	*B. pertussis*	*B. parapertussis*	*B. bronchiseptica*
Growth on blood free media	-	+	+
Motility	-	-	+
Urease	-	+	+
Citrate	-	+	+
Nitrate reduction	-	-	+

Treatment: Erythromycin is the drug of choice

Legionella pneumophila

Introduction:

Legionellosis was first recognized in association with the epidemic of legionnaires disease which occurred in Philadelphia in July 1976. Shortly following the epidemic, the causative agent was isolated from lung tissues of patient who died of this disease. The organism was named *Legionella pneumophila.*

ORGANISM:

- Gram negative rods
- Slow grower (2-5 days in enriched media containing L-cystein and supplemented source of ferric iron)
- Old culture may exhibit filamentous forms, swollen rods, and bizarre forms.
- Stain poorly with gram stain unless safranin is applied for long periods.
- Good growth obtained on Charcoal-Yeast Extract (CYE).
- Good growth occur in an atmosphere containing 2.5% CO2
- Soluble pigment that results in brown coloration on Feely-Gorman agar.
- Most strains are flagellated.

Biochemical Characteristics:

- Weak oxidase positive
- Strong catalase positive
- Liquefy gelatine
- No carbohydrate fermentation
- No nitrate reduction
- No urea hydrolysis
- Hydrolysis of hippurate Positive (This differentiate it from other legionella species)

Virulence factors:

1. Proteolytic enzymes
2. Exotoxin with lysed laboratory animal RBCs
3. Cytotoxin which caused inhibition of cell growth in laboratory animals.

Clinical Manifestation:
Acute pneumonia begins 2-10 days after exposure with a brief prodorm of malaise, myalgia, and headache, followed rapidly by prostration, high fever, an rigors. Cough, dyspnea, pleuritic and abdominal pain, vomiting, diarrhea, and unexplained encephalopathy are often seen.

Gram stain of sputum is not diagnostic. Leukocytosis, elevated ESR, proteinurea, hematuria and abnormal serum enzyme determination are common. The common causes of death due to respiratory failure and shock.

Transmission:
Via respiratory tract.

Collection & Processing of Speciemn:
Caution: Specimen must be handled in a biological safety cabinet.
1. Lung tissue obtained at autopsy or biopsy is optimally selected from areas of necrosis. A representative portion of specimen should be placed in 10% neutral formalin for Direct Fluorescent Antibody and histopathological examination.
2. Pleural fluid, transtracheal aspirate.

Diagnosis:
1. Isolation and identification of Legionella from clinical specimens either on artificial media or guinea pig inoculation followed by embryonated hen`s egg.
2. Demonstration of a four-fold or greater rise in antibody titer from acute phase to convalescent phase by the IFA test.
3. Demonstration of the organism in clinical specimen by DFA.

Treatment:
Erythromycin is the drug of choice.

BRUCELLA

• **Gram-negative bacilli**

Habitat

• ***Chronically infected domestic animals***

Medically Important Species

• ***B. abortus*** ***- Cattles***
• ***B. melitensis*** ***- Goats & sheeps***
• ***B. canis*** ***- Dogs***

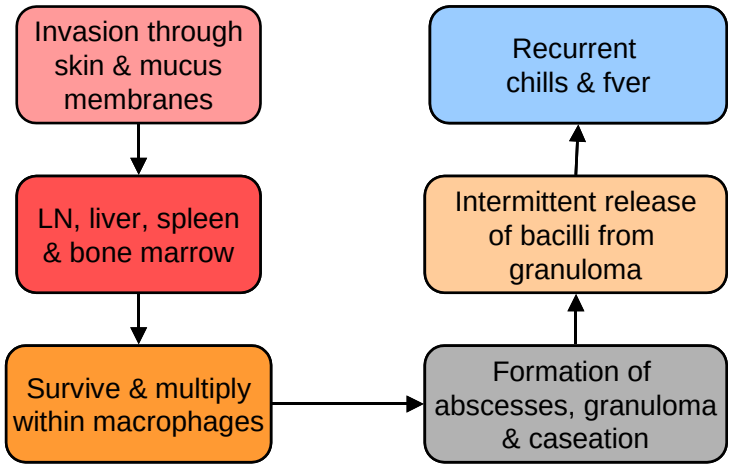
TRANSMISSION

• Primarily animal disease : Causes abortion & sterility

Transmission

• From animals to animals & humans by:
  ◦ Abrasion in skin
  ◦ Inhalation
  ◦ Ingestion of contaminated milk & cheese
• Persons at High Risk
  ◦ Dairy workers & farmers
  ◦ Live stock handlers & veternarians
  ◦ Slaughterhouse employees

PATHOGENESIS OF BRUCELLOSIS (Undulating fever)



- Infection of mammary glands of both animals& humans occur. Bacilli shed in milk
- Placental transfer & fetal infection occurs only in animals due to presence of erythritol in placental tissues

CLINICAL FEATURES OF BRUCELLOSIS (Undulating fever)

- Incubation period: 1-3 weeks
- Initially influenza-like
- Undulating (rising & falling) fever for weeks & months
- A chronic illness
- Splenomegaly
- Hepatomegaly
- Enlarged lymph nodes

LAB DIAGNOSIS

- Specimens
  Blood, LN & BM biopsy
- Culture
  Require 5-10% CO_2
- Serology
  - Antibody titre of 1:160 or more (significant)
  - Titre returns to normal within a year of successful treatment

TREATMENT

- **Ciprofloxacin**
  May be up to 6 weeks to prevent relapses

PREVENTION

- Pasteurization of milk
- Immunization of animals
- Eradication of infected livestock
- Minimize occupational exposure

Syphilis
Treponema pallidum

General Characteristics of the Organism:

1. A thin, motile spiral organism
2. Can not be cultivated on artificial media.
3. Can not be visualized by light microscope
4. Rabbits could be infected with this organism.

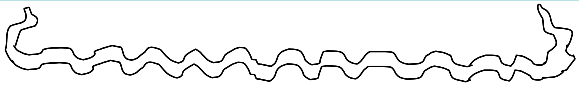
•Are spiral flexible organisms that move without flagella

•Multiply by transverse binary fission

Treponema : Many narrow regular coils

Borrelia : Few wide, irregular coils

Leptospira : Many very narrow coils with hooked ends



Clinical description of the disease:

Syphilis is a chronic granulomatous disease that characteristically progress by stages of clinically apparent infections. Each stage has different manifestation and lesion morphology, and stages are separated by periods of latency. The only evidence of infection is a reactive serologic test during the latency periods.

Except for congenital syphilis which is transmitted from infected mothers to the fetus across the placenta, syphilis is almost always transmitted by sexual exposure.

PRIMARY STAGE:

The primary syphilitic lesion, the chancre, appears. The chancre is most frequently a painless, ulcer-like lesion with raised borders and a necrotic base. The serum (exudate) from the chancre contains *T. pallidum* and is highly infectious. The chancre heals spontaneously without scaring over a period of 2-3 weeks.

SECONDARY STAGE:

It occur 6 weeks to 6 months after the chancre heals. Symptoms of this stage includes, headache and malaise, general lymphoadenopathy and a variety of skin rashes, hepatitis, meningitis, glmerulnephritis and nephritis. Nephrosis is a result of Antigen-antibody complexes deposited in the kidney. Secondary lesions heal spontenaneously over a period of several weeks and then enters in a latent stage number 2.

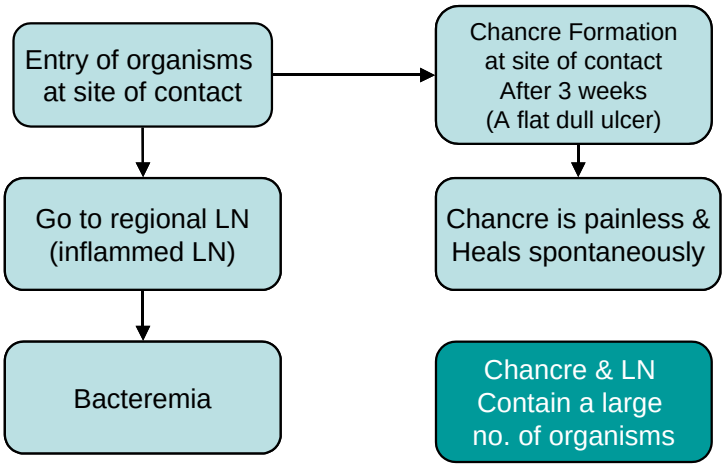
TERTIARY STAGE:

The destructive stage of the disease. It may begin 5-20 years after the initial infection. Only one third (1/3) of untreated patient develop tertiary which are of three types:

1. 80% are aneurysms (تمدد في الأوعية الدموية) : Leading to dilatation of aortic valve
2. 15% are CNS disease and meningitis
3. 5% gummas (الأورام الصمغية): Lesions which histologically resemble a tubercle. At this stage, bone and teeth deformation may occur.

CONGINITAL SYPHILIS:

The fetus may be aborted, stillborn or liveborn or without clinical evidence of syphilis. The lesions of early congenital syphilis are of the secondary type and may be present at birth. Blindness secondary to optic atrophy. Deformed bones may also occur.



LABORATORY IDENTIFICATION:

T. pallidum can not be cultivated in the laboratory In-Vitro. Only two methods are available for the diagnosis of *T. pallidum.*

1. DARK-FIELD MICROSCOPY: Most specific for infectious syphilis.

- Clean the chancre with gauze moistened with sterile sodium chloride solution. Dry it.
- Scrap the edges of the chancre several times with the flat side of a sterile lancet. Do not draw blood.
- Press with dry sterile gauze.
- Remove the swab and wait for a few minutes until a pinkish serous fluid appear. Draw off the fluid with a Pasteur pipette.
- Place a drop of the fluid on a thin glass slide.
- Examine under the microscope using dark field condenser. Search for the characteristic morphology of *T. pallidum*. (Motile thin spirochetes, rotating around their long axes.

2. SEROLOGICAL TESTING:

Two types of antigens are used.

A. Non-Treponemal Antigens Test: It utilizes **"cardiolipin"** as its antigen, which reacts with IgM or IgG immunoglobulin known as **"reagin"** antibody

1. Flocculation test (VDRL): (Venerial Disease Research Laboratories)

This test is based on the fact that particles of the lipid antigen (cardiolipin) remains dispersed in normal sera, but combines with reagin to form visible aggregate within a few minutes. Rapid Plasma Reagin test (RPR) is a good modification for rapid surveys.

2. Complement fixation test "Wasserman, Kolmer"

This test is based on the fact that, reagin-containing sera fix complement in the presence of cardiolipin.

NB: Biologic false results may occur with many patients having:

1. Malaria
2. Leprosy
3. Measles
4. Infectious mononucleosis
5. SLE

B. Treponemal Antibody Test:

1. Fluorescent Treponema Antibody Absorption Test (FTA-ABS)

This test employs indirect immunofluorescence (Killed *T. pallidum* + patient`s serum + labeled anti-human gammaglobulins). The test serum is added to the antigen (killed *T. pallidum*) which is fixed on a glass slide, incubated and then washed to remove excess serum. Conjugate (Labeled antihuman gammaglobulin) is added , incubated and then washed. This test is read microscopically with an ultraviolet light source.

2. *T. pallidum* immobilization test (TPI):

Specific antibodies in the patient serum after the second week of infection could be demonstrated by their ability to immobilize actively motile *T. pallidum*, extracted from the chancre of infected rabbits. The test is read microscopically with an dark field microscope.

3. *T. pallidum*-Hemagglutination test (TP-HA):

Red cells are treated to adsorb treponemal antigens on their surface. When mixed with serum containing antitreponemal antibodies, the cells become clumped. Clumping is considered as positive results.

NB: VDRL and FTA-ABS tests can also be performed on spinal fluids. Antibodies do not reach the CSF from the blood stream but are probably formed in CNS in response to syphylitic infection.

TREATMENT: Penicillin is the treatment of choice of syphilis

Diseases related to Treponema:

1. **Yaws**: (Frambesia): is a tropical infection of the skin, bones and joints caused by *T. pertenue*
2. **Pinta**

is a human skin disease endemic to Mexico, Central America, and South America. It is caused by infection with a spirochete, Treponema carateum, which is morphologically and serologically indistinguishable from the organism that causes syphilis.

Pinta initially presents as a raised papule, followed by a generalized eruption of flat, reddened areas, and is followed by the development of bluish coloration and a subsequent loss of pigmentation. Unlike syphilis, it is transmitted by nonsexual skin contact, often between children living in conditions of poor hygiene. The disease can be treated with **penicillin, tetracycline**, or **chloramphenicol**, and can be prevented through contact tracing by public health officials.

LEPTOSPIRA INTERROGANS

Causative agent of Leptospirosis : A zoonotic disease
Habitat : Infected domestic live-stock & pets
The organism settles in the kidney and excreted in urine

Mode of Infection

Direct Contact with:
Urine of infected animal
Water & soil recently contaminated with infected urine
The organism enters body through:
Skin lesions
Conjunctival mucus membrane
Ingestion

High-Risk Groups
Farmers
Sewer workers
LEPTOSPIROSIS
Pathogenesis
Incubation period : 1-2 weeks
Bacteremia : organisms multiply in liver, spleen, kidney, meninges, conjunctiva

Clinical Features
Influenza-like followed by hepatitis & meningitis
Weil's Disease (severe leptospirosis)
Jaundice, hemorrhage, renal failure
Lab Diagnosis
Dark field Microscopy of blood & CSF
Sero-diagnosis

Treatment & Prevention
Penicillin
Animal immunization
Proper treatment and disposal of contaminated water

BORRELIA RECURRENTIS

Can be stained with Giemsa stain in blood film
Can grow in media containing serum & tissue extracts
High frequency of antigenic variation in major surface protein and is responsible for :
Organism can escape immune system
Relapses of disease
Disease : Relapsing Fever

Transmission :
Tick-borne Relapsing Fever
From infected rodents to human
Louse-borne Relapsing Fever
From infected human to human

Clinical Features
Bacteremia : Infect various organs
A non-pruritic rash at bite site
Fever, rigors and headache for weeks to months
Weeks to months later: Cardiac and neurological symptoms
Predominant arthritis
One of the causes of PUO

Lab Diagnosis
Giemsa staining of blood smear
Detection of IgM OR rising titer of IgG
PCR

Treatment & Prevention
Amoxycillin
Louse, tick & rodent control
Hygienic measures

CHLAMYDIA

General Characteristics

- Obligate intracellular bacteria
- Have ribosomes like bacteria
- Are metabolically deficient

Morphology

- Small rounded organism
- Multiply by binary fission
- Cell wall consists of inner & outer membranes but differ from that of Gram-negative bacteria by absence of peptidoglycan

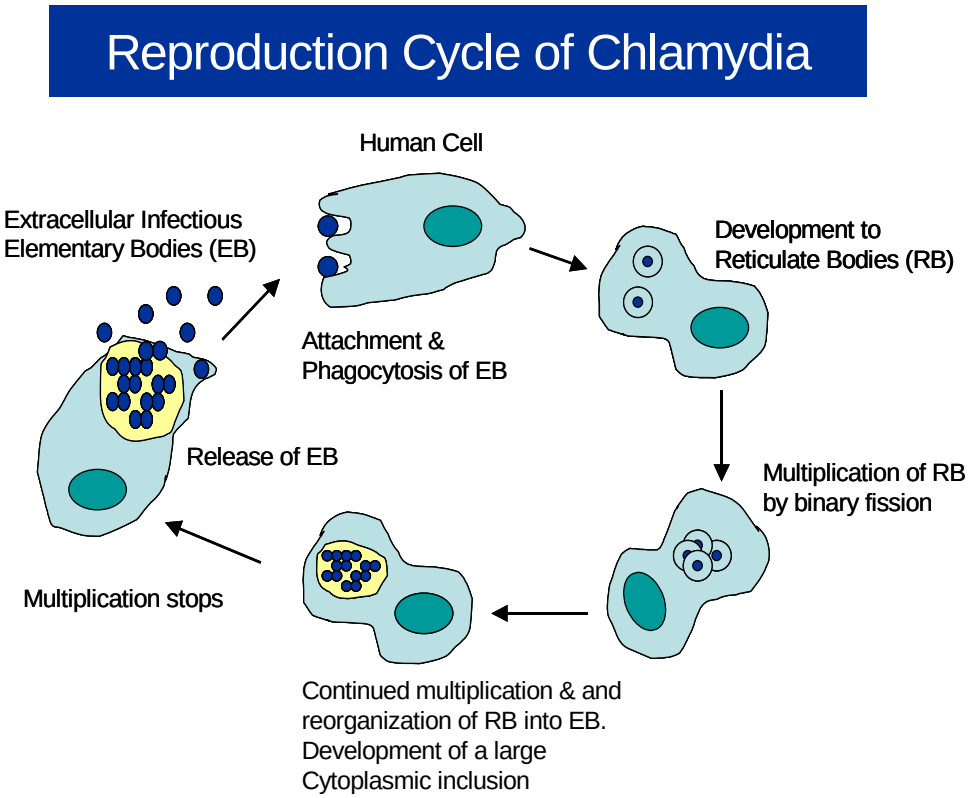
Important Species

C. psittaci
C. pneumoniae
C. trachomatis

Cultural Characters

Grow in:

Yolk sac of chicken embryo
Tissue culture (McCoy cells)



PATHOGENESIS

- Infect epithelial cells of mucous membranes & lungs
- Virulence is due to:
- Resistance to phagocytic killing by lysosomal enzymes
- Heat-labile toxin
- Competition with host cell for nutrients
- Host's immune response may account for inflammation & tissue destruction

CHLAMYDIA PSITTACI

A zoonotic respiratory disease
Natural habitat : birds
Transmitted through inhalation of :
Respiratory secretions & dust from faeces of infected birds
Common in poultry workers
Disease : Pneumonia (Psittacosis)
Diagnosis
Isolation of organism from sputum by tissue culture
Complement fixation test to detect specific Abs
Treatment
Tetracycline in adults
Erythromycin in babies

CHLAMYDIA PNEUMONIAE

Also known as TWAR
(TW – Taiwan & AR – acute respiratory)
Cause atypical pneumonia like *Mycoplasma pneumoniae*

Treatment
Tetracycline in adults
Erythromycin in babies

CHLAMYDIA TRACHOAMATIS

15 serotypes (A-L)
Transmission: Through close personal contact like:

- Sexual
- Passage through birth canal
- Finger to eye or fomite to eye (Trachoma)

DISEASES
1. Trachoma

- Caused by serotypes A, B, Ba & C
- One of the leading causes of blindness in developing countries with dry & hot weather
- Chronic conjunctivitis : leads to scarring of eye lids and cornea

2. Genital Tract Infections (Serotypes D-K)

- Non-gonococcal urethritis in men
- A common cause of non-gonococcal urethritis
- Mucopurrulent urethral discharge
- May progress to epidydmitis & orchitis
- Cervicitis & Vaginitis
- Mucopurrulent vaginal discharge
- Pelvic Inflammatory Disease (PID)
- May lead to secondary infertility

3. Neonatal Infections (Caused by serotypes D-K)

- Acquired from mother's birth canal
- Inclusion Conjunctivitis
- Profuse mucopurrulent discharge 7-12 days after birth
- Pneumonia

4. Lymphogranuloma Venereum (LGV)

- Caused by serotypes L1, L2 & L3
- A STD with lesions on genitalia & LNs (buboes)

5. Reiter's Disease

An autoimmune disease caused by Abs formed against *C. trachomatis* which cross react with antigens on cells of urethra & joints.

LAB DIAGNOSIS

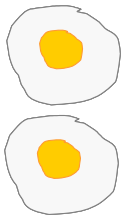
- Specimens from urethra, conjunctiva, sputum & cervix
- Microscopy
- Chlamydial "cytoplasmic inclusions" are detected by:
- Giemsa staining
- Fluorescent Ab staining
- PCR
- Cell Culture
- Sero-diagnosis

TREATMENT
Tetracycline in adults
Erythromycin or Azithromycin in babies

Calymmatobacterium granulomatous
GRANULOMA INGUINALE

General characteristics
Capsulated short Gram-negative rod
A STD with higher incidence in homosexuals
Clinical Features
Initially papules appear on external genitalia which ulcerate and extend widely – ulcer formation
Base of ulcer is "BEEFY"; spreads by contact so is known as "KISSING ulcers"
Lymph Nodes may enlarge
Treatment : Tetracycline

MYCOPLASMA



GENERAL CHARACTERISTICS
The smallest free-living organism (0.3 μ diameter)
Have no cell wall
Insensitive to penicillins & cephalosporins
Poorly stained by Gram-staining
Cytoplasmic membrane contains cholesterol
Slow growth on specialized artificial culture media (a week)
Typical **"fried-egg"** appearance of colonies by microscope

MYCOPLASMA PNEUMONIAE

MAIN DISEASE
- Primary atypical pneumonia
- Common in late summer and early autumn

PATHOGENESIS & EPIDEMIOLOGY
- Droplet infection
- Organism adhere to respiratory epithelium
- Inhibit ciliary motion
- Damage epithelium
- ~10% of infected individuals develop pneumonia
- 5-10% of community acquired pneumonia
- Common in chidren & young adults
- Increased incidence in winter

PRIMARY ATYPICAL PNEUMONIA

CLINICAL FEATURES
Sore throat, fever & headache
Cough with small amount of whitish non-purulent sputum
Some extrapulmonary symptoms
Opacities on chest X-Rays

IMMUNITY
Incomplete: second episode can occur
Auto IgM Abs are produced against type O RBCs
Agglutinate RBCs at 4 ^{o}C but not at 37 ^{o}C : are called "cold agglutinins"

UREAPLASMA URELYTICUM

Differentiated from mycoplasma due to urease enzyme production
Like mycoplasma produce "fried egg" colonies on specialized medium
Diseases
- Non-gonococcal, non-chlamydial urethritis in men
- Post-partum fever in women
- Transmitted by sexual contact

MYCOPLASMA & UREPLASMA
LAB DIAGNOSIS
Culture : "Fried egg" colonies on specialized medium
Cold Agglutinin detection
A titer of 1:128 or higher – indicates recent infection

TREATMENT
Tetracycline OR Spectinomycin

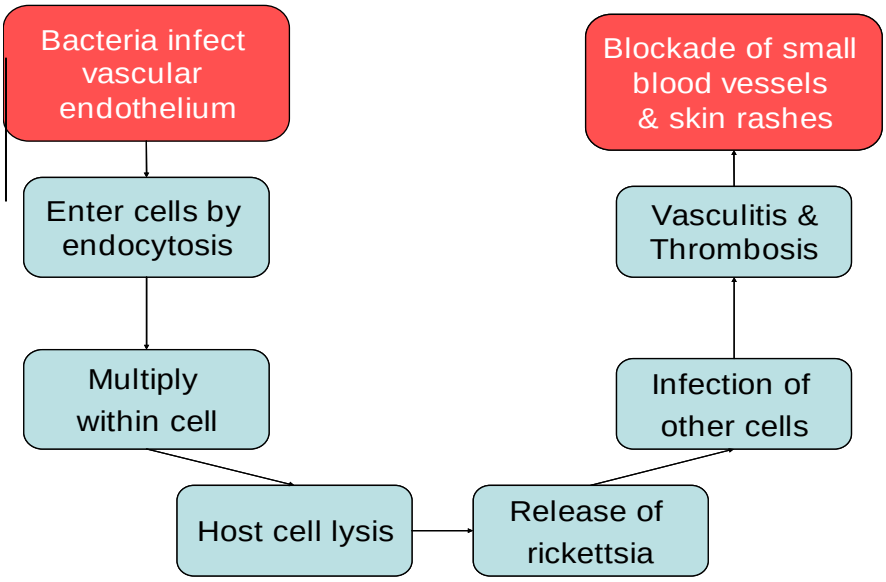
RICKETTSIA & COXIELLA

- Are obligate intracellular parasites
- Have animal reservoirs (zoonotic diseases)
- Humans are accidental host in most cases (except *R. prowazeki*)
- Are transmitted through vectors (except *C. burnetti*)

Morphology
- Coccobacilli
- Stained poorly with Gram-staining
- Can be stained within infected cells by Giemsa stain
- Multiply by binary fission

Growth characteristics
- Grow only in eukaryotic cell like
- Tissue cultures OR embryonated eggs
- Transmission
- Maintained in arthropods like ticks, lice, fleas & mite
- Transmitted to human by bite of arthropod vector (except *C. burnetti*)

PATHOGENICITY OF RICKETTSIA



Disease	

1. Rocky mountain Spotted fever	*R. rickttsii*	Ticks
2. Epidemic typhus	*R. prowazeki*	Lice
3. Q fever	*C. burnetti*	None

RICKETTSIA & COXIELLA : LAB DIAGNOSIS

- Serology
- PCR
- Tissue culture
- Weil-Felix Reaction
- Antigens of several species of Rickettsiae cross-react with cell wall O antigen of Proteus OX-2, OX-19, OX-K
- These Proteus antigens can be used in lab to detect presence of specific antibodies against certain Rickettsia in patients serum.
- Reaction negative in Q fever

TREATMENT

- Tetracycline
- Chloramphenicol

Basic Mycology

STRUCTURE & GROWTH

Because fungi (yeasts and molds) are **eukaryotic** organisms whereas bacteria are prokaryotic, they differ in several fundamental respects.

Two fungal cell structures are important medically:

1. The fungal cell wall consists primarily of chitin (not peptidoglycan as in bacteria); thus, fungi are insensitive to antibiotics, such as penicillin, that inhibit peptidoglycan synthesis.

   Chitin is a polysaccharide composed of long chains of *N*-acetylglucosamine. The fungal cell wall contains other polysaccharides as well, the most important of which is β-glucan, a long polymer of D-glucose. The medical importance of β-glucan is that it is the site of action of the antifungal drug caspofungin.

2. The fungal cell membrane contains ergosterol, in contrast to the human cell membrane, which contains cholesterol. The selective action of amphotericin B and azole drugs, such as fluconazole and ketoconazole, on fungi is based on this difference in membrane sterols.

Comparison of Fungi and Bacteria.

Feature	Fungi	Bacteria
Diameter	Approximately 4 μm (*Candida*)	Approximately 1 μm (*Staphylococcus*)
Nucleus	Eukaryotic	Prokaryotic
Cytoplasm	Mitochondria and endoplasmic reticulum present	Mitochondria and endoplasmic reticulum absent
Cell membrane	Sterols present	Sterols absent (except *Mycoplasma*)
Cell wall content	Chitin	Peptidoglycan
Spores	Sexual and asexual spores for reproduction	Endospores for survival, not for reproduction
Thermal dimorphism	Yes (some)	No
Metabolism	Require organic carbon; no obligate anaerobes	Many do not require organic carbon; many obligate anaerobes

There are two types of fungi: yeasts and molds.

1. **Yeasts** grow as **single cells** that reproduce by asexual budding.

2. **Molds** grow as **long filaments (hyphae)** and form a mat (**mycelium**).

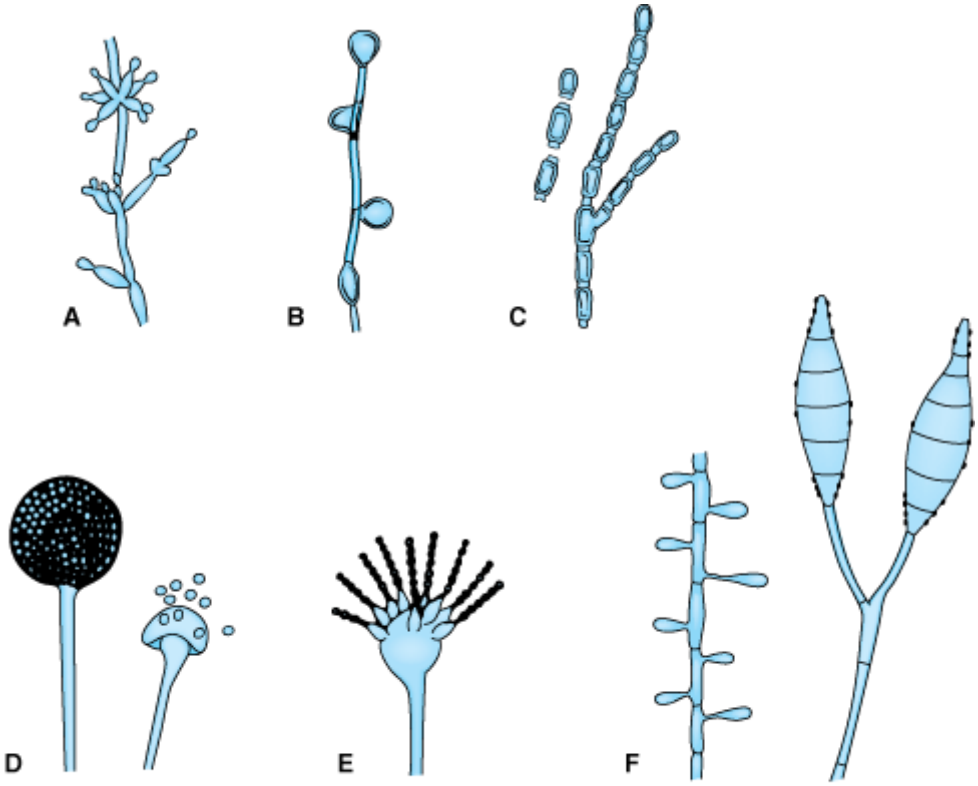
- Some hyphae form transverse walls (**septate hyphae**), whereas others do not (**nonseptate hyphae**). Nonseptate hyphae are multinucleated (coencytic).
- Several medically important fungi are thermally **dimorphic;** i.e., they form different structures at different temperatures.
- They exist as molds in the environment at ambient temperature and as yeasts (or other structures) in human tissues at body temperature.
- Most fungi are obligate aerobes; some are facultative anaerobes; but none are obligate anaerobes.
- All fungi require a preformed organic source of carbon—hence their frequent association with decaying matter.
- The natural habitat of most fungi is, therefore, the **environment.**
- An important exception is *Candida albicans,* which is part of the normal human flora.
- Some fungi reproduce sexually by mating and forming sexual spores, e.g., **zygospores, ascospores,** and **basidiospores.**
- Zygospores are single large spores with thick walls; ascospores are formed in a sac called ascus; and basidiospores are formed externally on the tip of a pedestal called a basidium.
- The classification of these fungi is based on their sexual spores. Fungi that do not form sexual spores are termed "imperfect" and are classified as **fungi imperfecti.**
- Most fungi of medical interest propagate asexually by forming **conidia** (asexual spores) from the sides or ends of specialized structures.
- The shape, color, and arrangement of conidia aid in the identification of fungi. Some important conidia are

(1) **arthrospores,** which arise by fragmentation of the ends of hyphae and are the mode of transmission of *Coccidioides immitis;*

(2) **chlamydospores,** which are rounded, thick-walled, and quite resistant (the terminal chlamydospores of *C. albicans* aid in its identification);

(3) **blastospores,** which are formed by the budding process by which yeasts reproduce asexually (some yeasts, e.g., *C. albicans,* can form multiple buds that do not detach, thus producing sausage like chains called **pseudohyphae,** which can be used for identification); and

(4) **sporangiospores,** which are formed within a sac (sporangium) on a stalk by molds such as*Rhizopus* and *Mucor.*



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Asexual spores. **A:** Blastoconidia and pseudohyphae (*Candida*). **B:** Chlamydospores (*Candida*). **C:** Arthrospores (*Coccidioides*). **D:** Sporangia and sporangiospores (*Mucor*). **E:** Microconidia (*Aspergillus*). **F:** Microconidia and macroconidia (*Microsporum*). (Modified and reproduced, with permission, from Conant NF et al: *Manual of Clinical Mycology,* 3rd ed. Saunders, 1971.)

- Although this book focuses on the fungi that are human pathogens, it should be remembered that fungi are used in the production of important foods, e.g., bread, cheese etc...
- Fungi are also responsible for the spoilage of certain foods.
- Because molds can grow in a drier, more acidic, and higher-osmotic-pressure environment than bacteria, they tend to be involved in the spoilage of fruits, grains, vegetables, and jams.

PATHOGENESIS

- The response to infection with many fungi is the formation of **granulomas.**
- Granulomas are produced in the major systemic fungal diseases, e.g., coccidioidomycosis, histoplasmosis, and blastomycosis, as well as several others.

- The cell-mediated immune response is involved in granuloma formation. Acute suppuration, characterized by the presence of neutrophils in the exudate, also occurs in certain fungal diseases such as aspergillosis and sporotrichosis.
- Fungi do not have endotoxin in their cell walls and do not produce bacterial-type exotoxins.
- Activation of the cell-mediated immune system results in a **delayed hypersensitivity skin test** response to certain fungal antigens injected intradermally.
- A positive skin test indicates exposure to the fungal antigen.
- It does *not* imply current infection, because the exposure may have occurred in the past.
- A negative skin test makes the diagnosis unlikely unless the patient is immunocompromised.
- Because most people carry *Candida* as part of the normal flora, skin testing with *Candida* antigens can be used to determine whether cell-mediated immunity is normal.

Transmission and Geographic Location of Some Important Fungi.

Genus	Habitat	Form of Organism Transmitted	Portal of Entry	Endemic Geographic Location
Coccidioides	Soil	Arthrospores	Inhalation into lungs	Southwestern United States and Latin America
Histoplasma	Soil (associated with bird feces)	Microconidia	Inhalation into lungs	Mississippi and Ohio River valleys in United States; many other countries
Blastomyces	Soil	Microconidia	Inhalation into lungs	States east of Mississippi River in United States; Africa
Paracoccidioides	Soil	Uncertain	Inhalation into lungs	Latin America
Cryptococcus	Soil (associated with pigeon feces)	Yeast	Inhalation into lungs	Worldwide
Aspergillus	Soil and	Conidia	Inhalation into	Worldwide

	vegetation		lungs	
Candida	Human body	Yeast	Normal flora of skin, mouth, gastrointestinal tract, and vagina	Worldwide

- Intact skin is an effective host defense against certain fungi (e.g., *Candida,* dermatophytes), but if the skin is damaged, organisms can become established.
- Fatty acids in the skin inhibit dermatophyte growth, and hormone-associated skin changes at puberty limit ringworm of the scalp caused by *Trichophyton.*
- The normal flora of the skin and mucous membranes suppress fungi.
- When the normal flora is inhibited, e.g., by antibiotics, overgrowth of fungi such as *C. albicans* can occur.
- In the respiratory tract, the important host defenses are the mucous membranes of the nasopharynx, which trap inhaled fungal spores, and alveolar macrophages.
- Circulating IgG and IgM are produced in response to fungal infection, but their role in protection from disease is uncertain.
- The cell-mediated immune response is protective; its suppression can lead to reactivation and dissemination of asymptomatic fungal infections and to disease caused by opportunistic fungi.

FUNGAL TOXINS & ALLERGIES

In addition to mycotic infections, there are two other kinds of fungal disease: (1) **mycotoxicoses,** caused by ingested toxins and

(2) **allergies** to fungal spores. The best-known mycotoxicosis occurs after eating *Amanita* mushrooms.

- These fungi produce five toxins, two of which—amanitin and phalloidin—are among the most potent hepatotoxins.
- The toxicity of amanitin is based on its ability to inhibit cellular RNA polymerase, which prevents mRNA synthesis.
- Another mycotoxicosis, ergotism, is caused by the mold *Claviceps purpura,* which infects grains and produces alkaloids (e.g., ergotamine and lysergic acid diethylamide [LSD]) that cause pronounced vascular and neurologic effects.

- Other ingested toxins, **aflatoxins,** are coumarin derivatives produced by *Aspergillus flavus* that cause liver damage and tumors in animals and are suspected of causing hepatic carcinoma in humans.
- Aflatoxins are ingested with spoiled grains and peanuts and are metabolized by the liver to the epoxide, a potent carcinogen.
- Aflatoxin B1 induces a mutation in the p53 tumor suppressor gene, leading to a loss of p53 protein and a consequent loss of growth control in the hepatocyte.
- Allergies to fungal spores, particularly those of *Aspergillus,* are manifested primarily by an asthmatic reaction (rapid bronchoconstriction mediated by IgE), eosinophilia, and a "wheal and flare" skin test reaction.
- These clinical findings are caused by an immediate hypersensitivity response to the fungal spores.

LABORATORY DIAGNOSIS

There are four approaches to the laboratory diagnosis of fungal diseases:

(1) Direct microscopic examination,

(2) Culture of the organism,

(3) DNA probe tests, and

(4) Serologic tests.

- Direct microscopic examination of clinical specimens such as sputum, lung biopsy material, and skin scrapings depends on finding characteristic asexual spores, hyphae, or yeasts in the light microscope.
- The specimen is either treated with 10% KOH to dissolve tissue material, leaving the alkali-resistant fungi intact, or stained with special fungal stains.

Some examples of diagnostically important findings made by direct examination are

(1) The spherules of *C. immitis* and

(2) The wide capsule of *Cryptococcus neoformans* seen in India ink preparations of spinal fluid.

- Calcofluor white is a fluorescent dye that binds to fungal cell walls and is useful in the identification of fungi in tissue specimens. Methenamine-silver stain is also useful in the microscopic diagnosis of fungi in tissue.
- Fungi are frequently cultured on Sabouraud's agar, which facilitates the appearance of the slow-growing fungi by inhibiting the growth of bacteria in the specimen.
- Inhibition of bacterial growth is due to the low pH of the medium and to the chloramphenicol and cycloheximide that are frequently added.

- The appearance of the mycelium and the nature of the asexual spores are frequently sufficient to identify the organism.
- Tests involving DNA probes can identify colonies growing in culture at an earlier stage of growth than can tests based on visual detection of the colonies.
- As a result, the diagnosis can be made more rapidly. At present, DNA probe tests are available for *Coccidioides, Histoplasma, Blastomyces,* and*Cryptococcus.*
- Tests for the presence of antibodies in the patient's serum or spinal fluid are useful in diagnosing systemic mycoses but less so in diagnosing other fungal infections.
- As is the case for bacterial and viral serologic testing, a significant rise in the antibody titer must be observed to confirm a diagnosis.
- The complement fixation test is most frequently used in suspected cases of coccidioidomycosis, histoplasmosis, and blastomycosis.
- In cryptococcal meningitis, the presence of the polysaccharide capsular antigens of *C. neoformans* in the spinal fluid can be detected by the latex agglutination test.

ANTIFUNGAL THERAPY

- The drugs used to treat bacterial diseases have no effect on fungal diseases.
- For example, penicillins and aminoglycosides inhibit the growth of many bacteria but do not affect the growth of fungi.
- This difference is explained by the presence of certain structures in bacteria, e.g., peptidoglycan and 70S ribosomes, that are absent in fungi.
- The most effective antifungal drugs, amphotericin B and the various azoles, exploit the presence of **ergosterol** in fungal cell membranes that is not found in bacterial or human cell membranes.
- Amphotericin B disrupts fungal cell membranes at the site of ergosterol and azole drugs inhibit the synthesis of ergosterol, which is an essential component of fungal membranes.
- Another antifungal drug, caspofungin (Cancidas), inhibits the synthesis of β-glucan, which is found in fungal cell walls but not in bacterial cell walls.
- Human cells do not have a cell wall.
- There is no clinically significant resistance to antifungal drugs.

Mechanism of Action and Adverse Effects of Antifungal Drugs.

Usage	Name of Drug	Mechanism of Action	Important Adverse Reactions
Systemic use (intravenous, oral)	Amphotericin B	Binds to ergosterol and disrupts fungal cell membranes	Renal toxicity, fever, and chills; monitor kidney function; use test dose; liposomal preparation reduces toxicity
	Azoles such as fluconazole, ketoconazole, itraconazole, voriconazole, posaconazole	Inhibits ergosterol synthesis	Ketoconazole inhibits human cytochrome P450; this decreases synthesis of gonadal steroids resulting in gynecomastia
	Echinocandins such as caspofungin, mycafungin	Inhibits synthesis of D-glucan, a component of fungal cell wall	Well tolerated
	Flucytosine (FC)	Inhibits DNA synthesis; FC converted to fluorouracil, which inhibits thymidine synthetase	Bone marrow toxicity
	Griseofulvin	Disrupts mitotic spindle by binding to tubulin	Liver toxicity
Topical use (skin only); too toxic for systemic use	Azoles such as cotrimazole, miconazole	Inhibits ergosterol synthesis	Well tolerated on skin
	Terbinafine	Inhibits ergosterol synthesis	Well tolerated on skin
	Tolnoftate	Inhibits ergosterol synthesis	Well tolerated on skin
	Nystatin	Binds to ergosterol and disrupts fungal cell membranes	Well tolerated on skin

CUTANEOUS & SUBCUTANEOUS MYCOSES:

INTRODUCTION

Medical mycoses can be divided into four categories:

(1) **Cutaneous,**

(2) **Subcutaneous,**

(3) **Systemic,**

(4) **Opportunistic.**

Features of Important Fungal Diseases.

Type	Anatomic Location	Representative Disease	Genus of Causative Organism(s)	Seriousness of Illness
Cutaneous	Dead layer of skin	Tinea versicolor	*Malassezia*	1 +
	Epidermis, hair, nails	Dermatophytosis (ringworm)	*Microsporum, Trichophyton, Epidermophyton*	2 +
Subcutaneous	Subcutis	Sporotrichosis	*Sporothrix*	2 +
		Mycetoma	Several genera	2 +
Systemic	Internal organs	Coccidioidomycosis	*Coccidioides*	4 +
		Histoplasmosis	*Histoplasma*	4 +
		Blastomycosis	*Blastomyces*	4 +
		Paracoccidioidomycosis	*Paracoccidioides*	4 +
Opportunistic	Internal organs	Cryptococcosis	*Cryptococcus*	4 +
		Candidiasis	*Candida*	2 + to 4 +
		Aspergillosis	*Aspergillus*	4 +
		Mucormycosis	*Mucor, Rhizopus*	4 +

1+, not serious, treatment may or may not be given; 2+, moderately serious, treatment often given; 4+, serious, treatment given especially in disseminated disease.

CUTANEOUS MYCOSES

Dermatophytoses

- Dermatophytoses are caused by fungi (**dermatophytes**) that infect only superficial keratinized structures (skin, hair, and nails), not deeper tissues.
- The most important dermatophytes are classified in three genera: *Epidermophyton, Trichophyton,* and *Microsporum.*
- They are spread from infected persons by direct contact. *Microsporum* is also spread from animals such as dogs and cats.
- This indicates that to prevent reinfection, the animal must be treated also.
- Dermatophytoses (tinea, ringworm) are chronic infections often located in the warm, humid areas of the body, e.g., athlete's foot.
- Typical ringworm lesions have an inflamed circular border containing papules and vesicles surrounding a clear area of relatively normal skin.
- Broken hairs and thickened broken nails are often seen.
- The disease is typically named for the affected body part, i.e., tinea capitis (head), tinea corporis (body), tinea cruris (groin), and tinea pedis (foot).
- *Trichophyton tonsurans* is the most common cause of outbreaks of tinea capitis in children and is the main cause of endothrix (inside the hair) infections.
- *Trichophyton rubrum* is also a very common cause of tinea capitis.
- *Trichophyton schoenleinii* is the cause of favus, a form of tinea capitis in which crusts are seen on the scalp.
- In some infected persons, hypersensitivity causes **dermatophytid ("id")** reactions, e.g., vesicles on the fingers. Id lesions are a response to circulating fungal antigens; the lesions do not contain hyphae.
- Patients with tinea infections show positive skin tests with fungal extracts, e.g., trichophytin.
- Scrapings of skin or nail placed in 10% KOH on a glass slide show septate hyphae under microscopy.
- Cultures on Sabouraud's agar at room temperature develop typical hyphae and conidia.
- Tinea capitis lesions caused by *Microsporum* species can be detected by seeing fluorescence when the lesions are exposed to ultraviolet light from a Wood's lamp.

- Treatment involves local antifungal creams (undecylenic acid, miconazole, tolnaftate, etc) or oral griseofulvin. Prevention centers on keeping skin dry and cool.

Tinea Versicolor

- Tinea versicolor (pityriasis versicolor), a superficial skin infection of cosmetic importance only, is caused by *Malassezia furfur.*
- The lesions are usually noticed as hypopigmented areas, especially on tanned skin in the summer.
- There may be slight scaling or itching, but usually the infection is asymptomatic. It occurs more frequently in hot, humid weather.
- The lesions contain both budding yeast cells and hyphae.
- Diagnosis is usually made by observing this mixture in KOH preparations of skin scrapings.
- Culture is not usually done.
- The treatment of choice is topical miconazole, but the lesions have a tendency to recur and a permanent cure is difficult to achieve.

Tinea Nigra

- Tinea nigra is an infection of the keratinized layers of the skin.
- It appears as a brownish spot caused by the melanin-like pigment in the hyphae.
- The causative organism, *Cladosporium werneckii,* is found in the soil and transmitted during injury.
- In the United States, the disease is seen in the southern states.
- Diagnosis is made by microscopic examination and culture of skin scrapings.
- The infection is treated with a topical keratolytic agent, e.g., salicylic acid.

SUBCUTANEOUS MYCOSES

These are caused by fungi that grow in soil and on vegetation and are introduced into subcutaneous tissue through **trauma.**

Sporotrichosis

- *Sporothrix schenckii* is a **dimorphic** fungus that lives on vegetation.

- When introduced into the skin, typically by a thorn, it causes a local pustule or ulcer with nodules along the draining lymphatics.
- There is little systemic illness. Lesions may be chronic. Sporotrichosis occurs most often in **gardeners, especially those who prune roses,** because they may be stuck by a rose thorn.
- In the clinical laboratory, round or cigar-shaped budding yeasts are seen in tissue specimens.
- In culture, hyphae occur bearing oval conidia in clusters at the tip of slender conidiophores (resembling a daisy).
- The drug of choice for skin lesions is itraconazole.
- It can be prevented by protecting skin when touching plants, moss, and wood.

Chromomycosis

- This is a slowly progressive granulomatous infection that is caused by several soil fungi (*Fonsecaea, Phialophora, Cladosporium,* etc) when introduced into the skin through trauma.
- These fungi are collectively called **dematiaceous** fungi, so named because their conidia or hyphae are dark-colored, either gray or black.
- Wartlike lesions with crusting abscesses extend along the lymphatics.
- The disease occurs mainly in the tropics and is found on bare feet and legs.
- In the clinical laboratory, dark brown, round fungal cells are seen in leukocytes or giant cells.
- The disease is treated with oral flucytosine or thiabendazole, plus local surgery.

Mycetoma

- Soil organisms (*Petriellidium, Madurella*) enter through wounds on the feet, hands, or back and cause abscesses, with pus discharged through sinuses.
- The pus contains compact colored granules.
- Actinomycetes such as *Nocardia* can cause similar lesions (actinomycotic mycetoma). Sulfonamides may help the actinomycotic form.
- There is no effective drug against the fungal form; surgical excision is recommended.

SYSTEMIC MYCOSES

INTRODUCTION

- These infections result from **inhalation** of the spores of **dimorphic** fungi that have their **mold** forms in the **soil.** Within the **lungs,** the spores differentiate into **yeasts** or other specialized forms.
- Most lung infections are asymptomatic and self-limited.
- However, in some persons, disseminated disease develops in which the organisms grow in other organs, cause destructive lesions, and may result in death.
- Infected persons do *not* communicate these diseases to others.

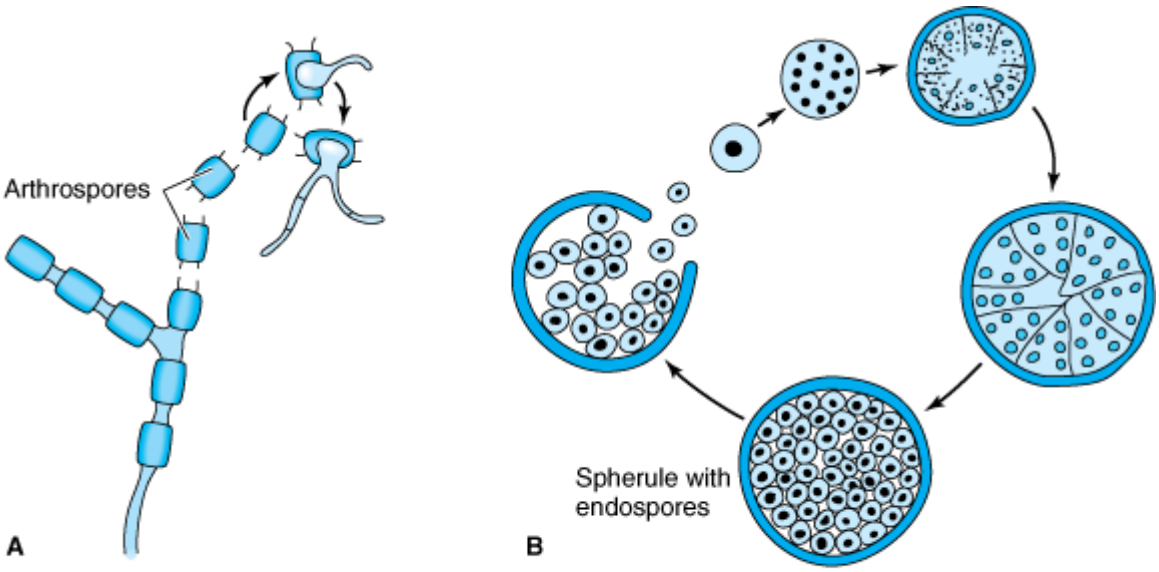
COCCIDIOIDES

Disease

- *Coccidioides immitis* causes coccidioidomycosis.

Properties

- *C. immitis* is a **dimorphic** fungus that exists as a **mold** in soil and as a **spherule** in tissue.



Stages of *Coccidioides immitis*.**A:** Arthrospores form at the ends of hyphae in the soil. They germinate in the soil to form new hyphae. If inhaled, the arthrospores differentiate into spherules. **B:** Endospores form within spherules in tissue. When spherules rupture, endospores disseminate and form new spherules. (Modified and reproduced, with permission, from Brooks GF et al: *Medical Microbiology,* 20th ed. Originally published by Appleton & Lange. Copyright © 1995 by The McGraw-Hill Companies, Inc.)

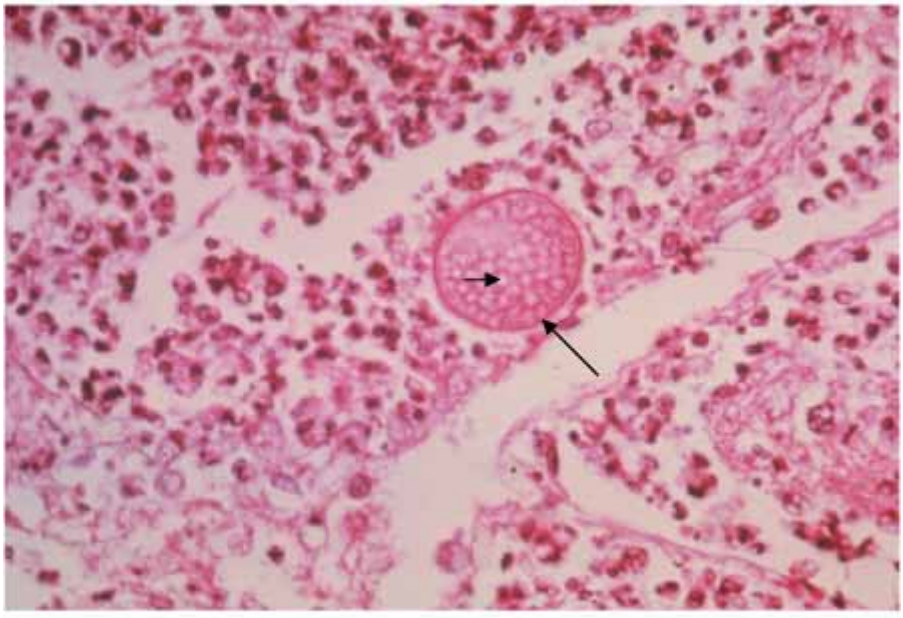
Transmission & Epidemiology

- The fungus is **endemic** in arid regions of the **southwestern United States** and **Latin America.**
- People who live in Central and Southern California, Arizona, New Mexico, Western Texas, and Northern Mexico, a geographic region called the Lower Sonoran Life Zone, are often infected.
- In soil, it forms hyphae with alternating **arthrospores** and empty cells.
- Arthrospores are very light and are carried by the wind.

They can be **inhaled** and infect the lungs.

Pathogenesis

- In the lungs, arthrospores form **spherules** that are large (30 µm in diameter), have a thick, doubly refractive wall, and are filled with **endospores**.
- Upon rupture of the wall, endospores are released and differentiate to form new spherules.
- The organism can spread within a person by direct extension or via the bloodstream.
- Granulomatous lesions can occur in virtually any organ but are found primarily in bones and the central nervous system (meningitis).



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Coccidioides immitis—Spherule. Long arrow points to a spherule in lung tissue. Spherules are large thick-walled structures containing many endospores. Short arrow points to an endospore. Provider: CDC/Dr. L. Georg.

- **Dissemination** from the lungs to other organs occurs in people who have a defect in cell-mediated immunity.
- Most people who are infected by *C. immitis*develop a cell-mediated (delayed hypersensitivity) immune response that restricts the growth of the organism.
- One way to determine whether a person has produced adequate cell-mediated immunity to the organism is to do a skin test.
- In general, a person who has a positive skin test reaction has developed sufficient immunity to prevent disseminated disease from occurring.
- If, at a later time, a person's cellular immunity is suppressed by drugs or disease, disseminated disease can occur.

Clinical Findings

- Infection of the lungs is often asymptomatic and is evident only by a positive skin test and the presence of antibodies.
- Some infected persons have an influenzalike illness with fever and cough. About 50% have changes in the lungs (infiltrates, adenopathy, or effusions) as seen on chest x-ray, and 10% develop erythema nodosum (see below) or arthralgias.
- This syndrome is called "valley fever" (in the San Joaquin Valley of California) or "desert rheumatism" (in Arizona); it tends to subside spontaneously.
- Disseminated disease can occur in almost any organ; the meninges, bone, and skin are important sites.
- The overall incidence of dissemination in persons infected with *C. immitis* is 1%, although the incidence in Filipinos and African Americans is 10 times higher.
- Women in the third trimester of pregnancy also have a markedly increased incidence of dissemination.
- Erythema nodosum (EN) manifests as red, tender nodules ("desert bumps") on extensor surfaces such as the shins.
- It is a delayed (cell-mediated) hypersensitivity response to fungal antigens and thus is an indicator of a good prognosis.
- There are no organisms in these lesions; they are not a sign of disseminated disease.
- EN is not specific for coccidioidomycosis; it occurs in other granulomatous diseases, e.g., histoplasmosis, tuberculosis, and leprosy.

- In infected persons, **skin tests** with fungal extracts (coccidioidin or spherulin) cause at least a 5-mm induration 48 hours after injection (delayed hypersensitivity reaction).
- Skin tests become positive within 2–4 weeks of infection and remain so for years but are often negative (anergy) in patients with disseminated disease.

Laboratory Diagnosis

- In tissue specimens, spherules are seen microscopically.
- Cultures on Sabouraud's agar incubated at 25°C show hyphae with arthrospores. (*Caution:*Cultures are highly infectious; precautions against inhaling arthrospores must be taken.)
- In serologic tests, IgM and IgG precipitins appear within 2–4 weeks of infection and then decline in subsequent months.
- Complement-fixing antibodies occur at low titer initially, but the titer rises greatly if dissemination occurs.

Treatment & Prevention

- No treatment is needed in asymptomatic or mild primary infection.
- Amphotericin B (Fungizone) or itraconazole is used for persisting lung lesions or disseminated disease. Ketoconazole is also effective in lung disease.
- If meningitis occurs, fluconazole is the drug of choice. Intrathecal amphotericin B may be required and may induce remission, but long-term results are often poor.
- There are no means of prevention except avoiding travel to endemic areas.

HISTOPLASMA

Disease

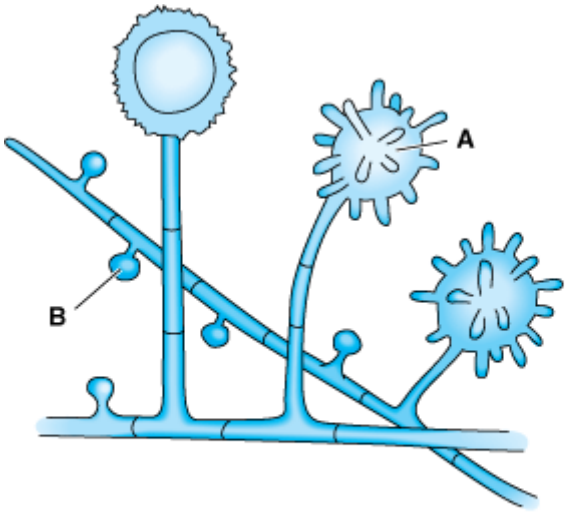
- *Histoplasma capsulatum* causes histoplasmosis.

Properties

- *H. capsulatum* is a **dimorphic** fungus that exists as a **mold** in soil and as a **yeast** in tissue. It forms two types of asexual spores:

(1)**tuberculate macroconidia,** with typical thick walls and fingerlike projections that are important in laboratory identification, and

(2) **microconidia,** which are smaller, thin, smooth-walled spores that, if inhaled, transmit the infection.



Asexual spores of *Histoplasma capsulatum.***A:** Tuberculate macroconidia. **B:** Microconidia. (Reproduced, with permission, from Brooks GF et al: *Medical Microbiology,*19th ed. Originally published by Appleton & Lange. Copyright © 1991 by The McGraw-Hill Companies, Inc.)

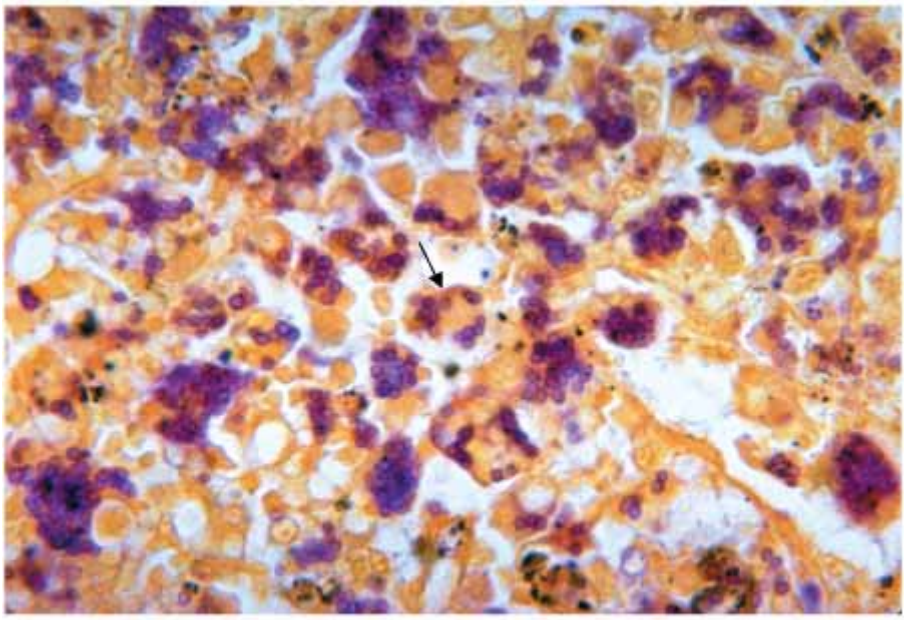
Transmission & Epidemiology

- This fungus occurs in many parts of the world. In the United States it is **endemic** in central and eastern states, especially in the **Ohio and Mississippi River valleys.**
- It grows in soil, particularly if the soil is heavily contaminated with **bird droppings,** especially from starlings.
- Although the birds are not infected, bats can be infected and can excrete the organism in their guano.
- In areas of endemic infection, excavation of the soil during construction or exploration of bat-infested caves has resulted in a significant number of infected individuals.
- In several tropical African countries, histoplasmosis is caused by *Histoplasma duboisii.*
- The clinical picture is different from that caused by *H. capsulatum.*

Pathogenesis & Clinical Findings

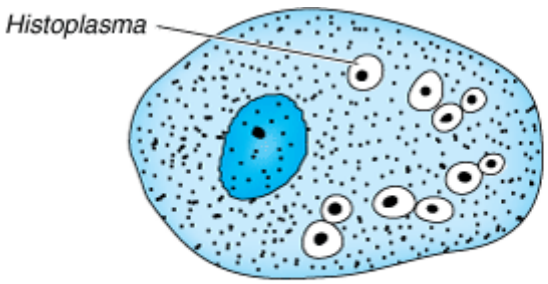
- **Inhaled spores** are engulfed by **macrophages** and develop into yeast forms.
- In tissues, *H. capsulatum* occurs as an **oval budding yeast inside macrophages**.

- The yeasts survive within the phagolysosome of the macrophage by producing alkaline substances, such as bicarbonate and ammonia, that raise the pH and thereby inactivate the degradative enzymes of the phagolysosome.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Histoplasma capsulatum—Yeasts within macrophages. Arrow points to a macrophage containing several purple-stained yeasts in the cytoplasm. Yeasts within macrophages can be seen in many macrophages in this specimen of spleen. Provider: CDC/Dr. M. Hicklin.



Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Histoplasma capsulatum. Yeasts are located within the macrophage. (Reproduced, with permission, from Brooks GF et al: *Medical Microbiology,* 19th ed. Originally published by Appleton & Lange. Copyright © 1991 by The McGraw-Hill Companies, Inc.)

- The organisms spread widely throughout the body, especially to the liver and spleen, but most infections remain asymptomatic, and the small granulomatous foci heal by calcification.
- With intense exposure (e.g., in a chicken house or bat-infested cave), pneumonia may become clinically manifest.
- Severe disseminated histoplasmosis develops in a small minority of infected persons, especially infants and individuals with reduced cell-mediated immunity, such as AIDS patients.
- In AIDS patients, ulcerated lesions on the tongue are typical of disseminated histoplasmosis.
- In immunocompetent people, EN can occur (see description of EN in Coccidioides above).
- EN is a sign that cell-mediated immunity is active and the organism will probably be contained.
- A skin test using histoplasmin (a mycelial extract) becomes positive, i.e., shows at least 5 mm of induration, within 2–3 weeks after infection and remains positive for many years.
- However, because there are many false-positive reactions (due to cross-reactivity) and many false-negative reactions (in disseminated disease), the skin test is not useful for diagnosis.
- Furthermore, the skin test can stimulate an antibody response and confuse the serologic tests.
- The skin test is useful for epidemiologic studies, and up to 90% of individuals have positive results in areas of endemic infection.

Laboratory Diagnosis

- In tissue biopsy specimens or bone marrow aspirates, oval **yeast cells within macrophages** are seen microscopically.
- Cultures on Sabouraud's agar show hyphae with tuberculate macroconidia when grown at low temperature, e.g., 25°C and yeasts when grown at 37°C.
- Tests that detect *Histoplasma*antigens by radioimmunoassay and *Histoplasma* RNA with DNA probes are also useful.
- In immunocompromised patients with disseminated disease, tests for antigens in the urine are especially useful because antibody tests may be negative.
- Two serologic tests are useful for diagnosis: complement fixation (CF) and immunodiffusion (ID).

- An antibody titer of 1:32 in the CF test with yeast phase antigens is considered to be diagnostic.
- However, cross-reactions with other fungi, especially *Blastomyces*, occur.

Treatment & Prevention

- No therapy is needed in asymptomatic or mild primary infections.
- With progressive lung lesions, oral itraconazole is beneficial.
- In disseminated disease, amphotericin B is the treatment of choice.
- In meningitis, fluconazole is often used because it penetrates the spinal fluid well.
- Oral itraconazole is used to treat pulmonary or disseminated disease, as well as for chronic suppression in patients with AIDS.
- There are no means of prevention except avoiding exposure in areas of endemic infection.

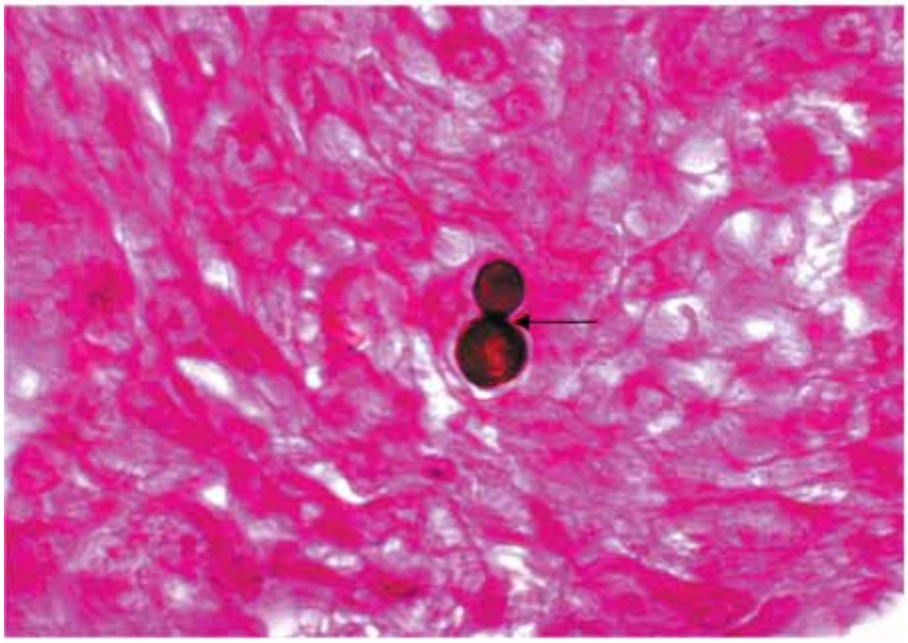
BLASTOMYCES

Disease

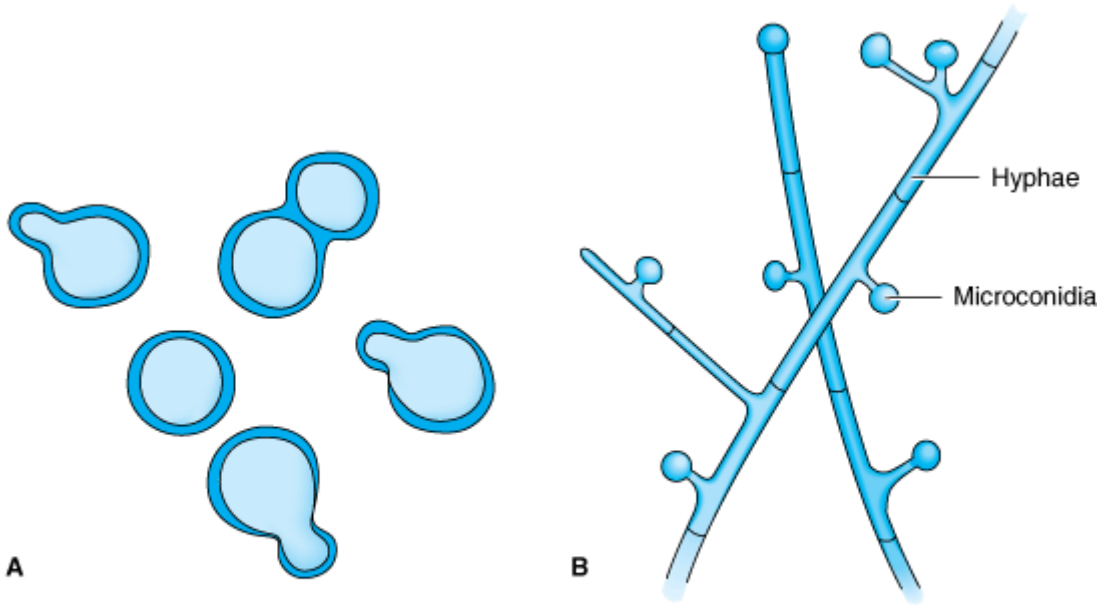
- *Blastomyces dermatitidis* causes blastomycosis, also known as North American blastomycosis.

Properties

- *B. dermatitidis* is a **dimorphic** fungus that exists as a mold in soil and as a yeast in tissue.
- The yeast is round with a doubly refractive wall and a single**broad-based bud**.
- Note that this organism forms a broad-based bud, whereas *Cryptococcus neoformans* is a yeast that forms a narrow-based bud.



Blastomyces dermatitidis—Broad-based budding yeast. Arrow points to the broad base of the budding yeast. Provider: CDC/Dr. L. Ajello.



*Blastomyces dermatitidis.***A:** Yeast with a broad-based bud at 37°C. **B:** Mold with microconidia at 20°C. (Reproduced, with permission, from Brooks GF et al: *Medical Microbiology,* 19th ed. Originally published by Appleton & Lange. Copyright © 1991 by The McGraw-Hill Companies, Inc.)

Transmission & Epidemiology

- This fungus is **endemic** primarily in eastern North America, especially in the region bordering the Ohio, Mississippi, and St. Lawrence rivers, and the Great Lakes region.
- Less commonly, blastomycosis has also occurred in Central and South America, Africa, and the Middle East.
- It grows in moist soil rich in organic material, forming hyphae with small pear-shaped conidia.
- Inhalation of the conidia causes human infection.

Pathogenesis & Clinical Findings

- Infection occurs mainly via the respiratory tract.
- Asymptomatic or mild cases are rarely recognized.
- Dissemination may result in ulcerated granulomas of skin, bone, or other sites.

Laboratory Diagnosis

- In tissue biopsy specimens, thick-walled yeast cells with single broad-based buds are seen microscopically.
- Hyphae with small pear-shaped conidia are visible on culture.
- The skin test lacks specificity and has little value.
- Serologic tests have little value.

Treatment & Prevention

- Itraconazole is the drug of choice for most patients, but amphotericin B should be used to treat severe disease.
- Surgical excision may be helpful.
- There are no means of prevention.

PARACOCCIDIOIDES

Disease

- *Paracoccidioides brasiliensis* causes paracoccidioidomycosis, also known as South American blastomycosis.

Properties

- *P. brasiliensis* is a **dimorphic** fungus that exists as a mold in soil and as a yeast in tissue.
- The yeast is thick-walled with **multiple buds,** in contrast to *B. dermatitidis,* which has a single bud.

Paracoccidioides brasiliensis. Note the multiple buds of the yeast form of *Paracoccidioides,* in contrast to the single bud of *Blastomyces.*

Transmission & Epidemiology

- This fungus grows in the soil and is endemic in rural Latin America.
- Disease occurs only in that region.

Pathogenesis & Clinical Findings

- The spores are **inhaled,** and early lesions occur in the lungs.
- Asymptomatic infection is common.
- Alternatively, oral mucous membrane lesions, lymph node enlargement, and sometimes dissemination to many organs develop.

Laboratory Diagnosis

- In pus or tissues, yeast cells with multiple buds are seen microscopically.
- A specimen cultured for 2–4 weeks may grow typical organisms.
- Skin tests are rarely helpful. Serologic testing shows that when significant antibody titers (by immunodiffusion or complement fixation) are found, active disease is present.

Treatment & Prevention

- The drug of choice is itraconazole taken orally for several months.
- There are no means of prevention.

OPPORTUNISTIC MYCOSES

INTRODUCTION

- Opportunistic fungi fail to induce disease in most immunocompetent persons but can do so in those with **impaired** host defenses.

CANDIDA

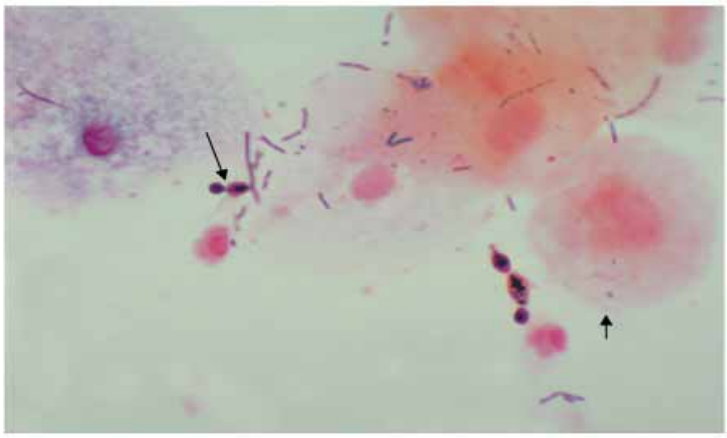
Diseases

- *Candida albicans,* the most important species of *Candida,* causes thrush, vaginitis, esophagitis, and chronic mucocutaneous candidiasis.

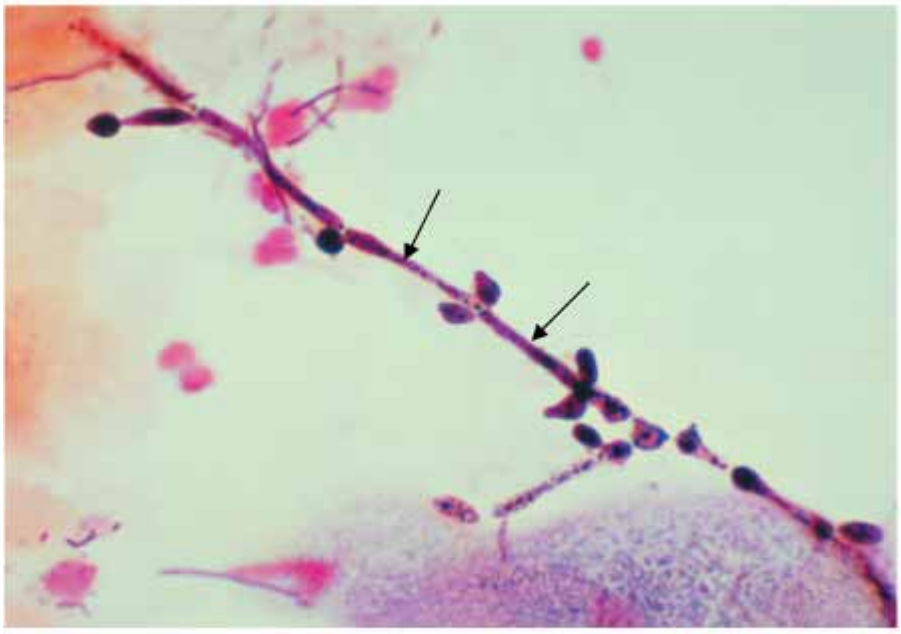
- It also causes disseminated infections such as right-sided endocarditis (especially in intravenous drug users) and blood stream infections (candidemia).
- Infections related to indwelling intravenous and urinary catheters are also important.

Properties

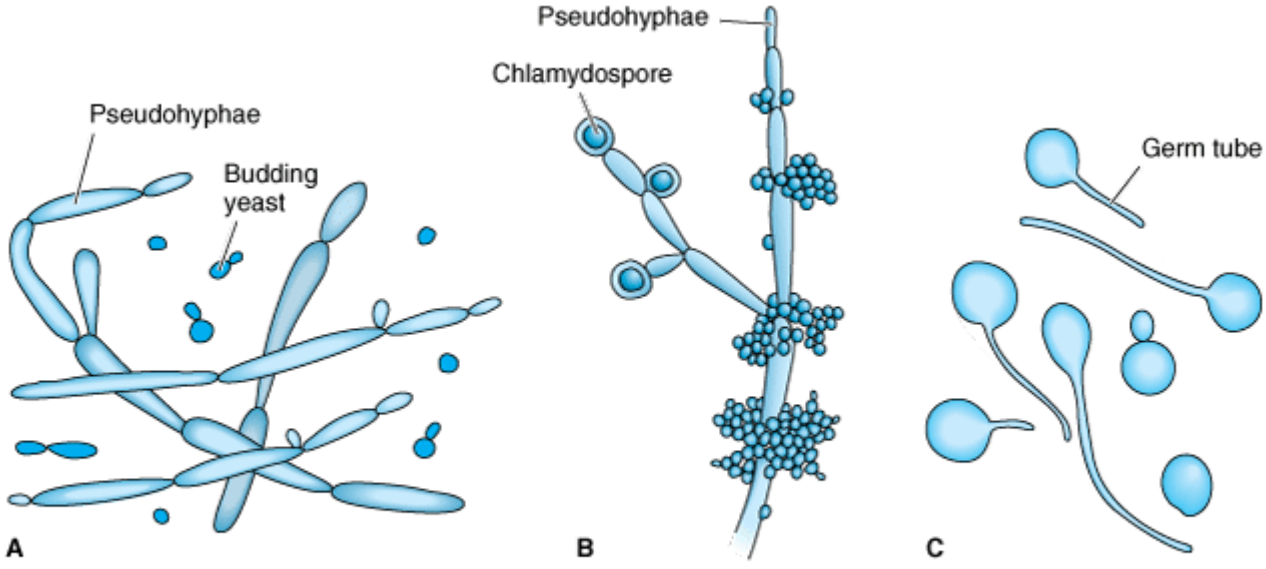
- *C. albicans* is an **oval yeast with a single bud**.
- It is part of the **normal flora** of mucous membranes of the upper respiratory, gastrointestinal, and female genital tracts.
- In tissues it may appear as yeasts or as **pseudohyphae**
- Pseudohyphae are elongated yeasts that visually resemble hyphae but are not true hyphae.
- Carbohydrate fermentation reactions differentiate it from other species, e.g., *Candida tropicalis, Candida parapsilosis, Candida krusei,* and *Candida glabrata.*



Candida albicans—Yeast. Long arrow points to a budding yeast. Short arrow points to the outer membrane of a vaginal epithelial cell. In this gram-stained specimen, various bacteria that are part of the normal flora of the vagina can be seen. Provider: CDC/Dr. S. Brown.



Candida albicans—Pseudohyphae. Two arrows point to pseudohyphae of *Candida albicans*. Provider: CDC/Dr. S. Brown.



*Candida albicans.***A:** Budding yeasts and pseudohyphae in tissues or exudate. **B:** Pseudohyphae and chlamydospores in culture at 20°C. **C:** Germ tubes at 37°C. (Reproduced, with permission, from Brooks GF et al: *Medical Microbiology*, 20th ed. Originally published by Appleton & Lange. Copyright © 1995 by The McGraw-Hill Companies, Inc.)

Transmission

- As a member of the normal flora, it is already present on the skin and mucous membranes.
- It is, therefore, not transmitted.
- The presence of *C. albicans*on the skin predisposes to infections involving instruments that penetrate the skin, such as needles (intravenous drug use) and indwelling catheters.

Pathogenesis & Clinical Findings

- When local or systemic host defenses are impaired, disease may result.
- Overgrowth of *C. albicans* in the mouth produces white patches called thrush. (Note that thrush is a "pseudomembrane,"
- Vulvovaginitis with itching and discharge is favored by high pH, diabetes, or use of antibiotics.
- Skin invasion occurs in warm, moist areas, which become red and weeping.
- Fingers and nails become involved when repeatedly immersed in water; persons employed as dishwashers in restaurants and institutions are commonly affected.
- Thickening or loss of the nail can occur.
- In immunosuppressed individuals, *Candida* may disseminate to many organs or cause chronic mucocutaneous candidiasis.
- Intravenous drug abuse, indwelling intravenous catheters, and hyperalimentation also predispose to disseminated candidiasis, especially right-sided endocarditis.
- *Candida* esophagitis, often accompanied by involvement of the stomach and small intestine, is seen in patients with leukemia and lymphoma.
- Subcutaneous nodules are often seen in neutropenic patients with disseminated disease.
- *C. albicans* is the most common species to cause disseminated disease in these patients, but *C. tropicalis* and *C. parapsilosis* are important pathogens also.

Laboratory Diagnosis

- In exudates or tissues, budding yeasts and pseudohyphae appear gram-positive and can be visualized using calcofluor-white staining.
- In culture, typical yeast colonies are formed that resemble large staphylococcal colonies.

- **Germ tubes** form in serum at 37°C, which serves to distinguish *C. albicans* from most other *Candida* species.
- **Chlamydospores** are typically formed by *C. albicans* but not by other species of *Candida.* Serologic testing is rarely helpful.
- **Skin tests** with *Candida* antigens are uniformly positive in immunocompetent adults and are used as an indicator that the person can mount a cellular immune response.
- A person who does not respond to *Candida* antigens in the skin test is presumed to have deficient cell-mediated immunity.
- Such a person is **anergic,** and other skin tests cannot be interpreted.
- Thus, if a person has a negative *Candida* skin test, a negative PPD skin test for tuberculosis could be a false-negative result.

Treatment & Prevention

- The drug of choice for oropharyngeal or esophageal thrush is fluconazole.
- Caspofungin or micafungin can also be used for esophageal candidiasis.
- Treatment of skin infections consists of topical antifungal drugs, e.g., clotrimazole or nystatin. Mucocutaneous candidiasis can be controlled by ketoconazole.
- Treatment of disseminated candidiasis consists of either amphotericin B or fluconazole.
- These two drugs can be used with or without flucytosine.
- Treatment of candidal infections with antifungal drugs should be supplemented by reduction of predisposing factors.
- Certain candidal infections, e.g., thrush, can be prevented by oral clotrimazole troches or nystatin "swish and swallow."
- Fluconazole is useful in preventing candidal infections in high-risk patients, such as those undergoing bone marrow transplantation and premature infants. Micafungin can also be used.
- There is no vaccine.

CRYPTOCOCCUS

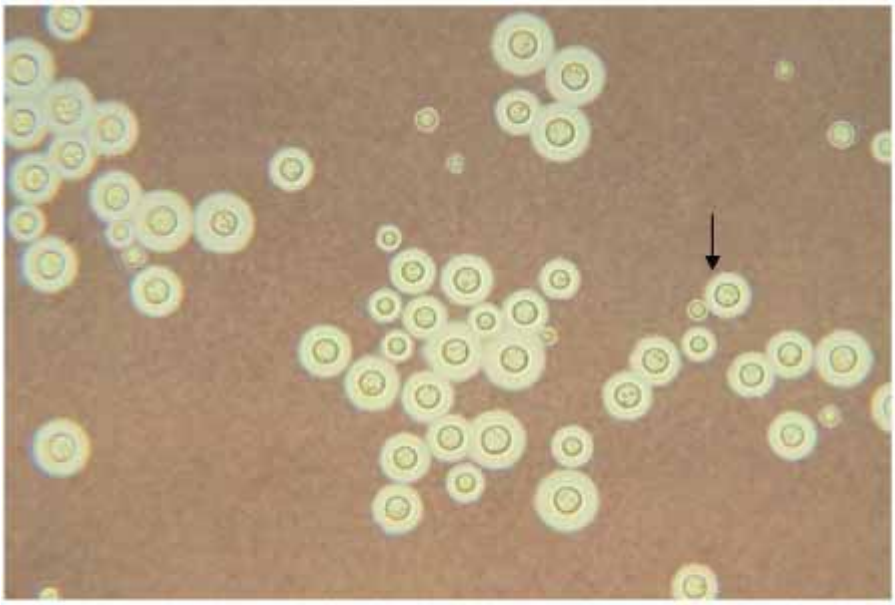
Disease

- *Cryptococcus neoformans* causes cryptococcosis, especially cryptococcal meningitis.

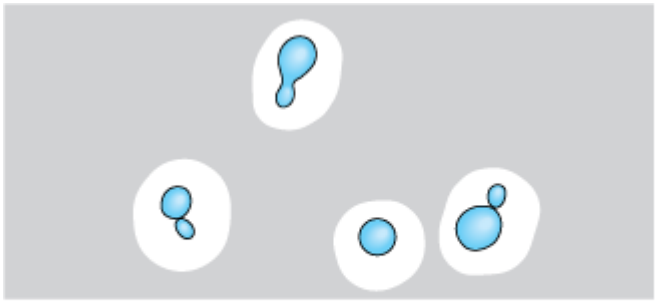
- Cryptococcosis is the most common life-threatening fungal disease in AIDS patients.

Properties

- *C. neoformans* is an **oval, budding yeast** surrounded by a **wide polysaccharide capsule**.
- It is not dimorphic. Note that this organism forms a narrow-based bud, whereas the yeast form of *Blastomyces dermatitidis* forms a broad-based bud.



Cryptococcus neoformans—India ink preparation. Arrow points to a budding yeast of *Cryptococcus neoformans*. Note the thick, translucent polysaccharide capsule outlined by the dark India ink particles. Provider: CDC/Dr. L. Haley.



Cryptococcus neoformans. India ink preparation shows budding yeasts with a wide capsule. India ink forms a dark background; it does not stain the yeast itself. (Reproduced, with permission, from Brooks GF et al: *Medical Microbiology*, 20th ed. Originally published by Appleton & Lange. Copyright © 1995 by The McGraw-Hill Companies, Inc.)

Transmission

- This yeast occurs widely in nature and grows abundantly in **soil containing bird (especially pigeon) droppings.**
- The birds are not infected. Human infection results from **inhalation** of the organism.
- There is no human-to-human transmission.

Pathogenesis & Clinical Findings

- Lung infection is often asymptomatic or may produce pneumonia.
- Disease occurs mainly in patients with reduced cell-mediated immunity, especially AIDS patients, in whom the organism disseminates to the central nervous system (meningitis) and other organs.
- Subcutaneous nodules are often seen in disseminated disease.
- Note, however, that roughly half the patients with cryptococcal meningitis fail to show evidence of immunosuppression.

Laboratory Diagnosis

- In spinal fluid mixed with **India ink,** the yeast cell is seen microscopically surrounded by a wide, unstained capsule.
- Appearance of the organism in Gram stain is unreliable, but stains such as methenamine-silver, periodic acid–Schiff, and mucicarmine will allow the organism to be visualized.
- The organism can be cultured from spinal fluid and other specimens.
- The colonies are highly mucoid, a reflection of the large amount of capsular polysaccharide produced by the organism.

- Serologic tests can be done for both antibody and antigen. In infected spinal fluid, **capsular antigen** occurs in high titer and can be detected by the **latex particle agglutination test.**
- This test is called the cryptococcal antigen test, often abbreviated as "crag."

Treatment & Prevention

- Combined treatment with amphotericin B and flucytosine is used in meningitis and other disseminated disease.
- There are no specific means of prevention.
- Fluconazole is used in AIDS patients for long-term suppression of cryptococcal meningitis.

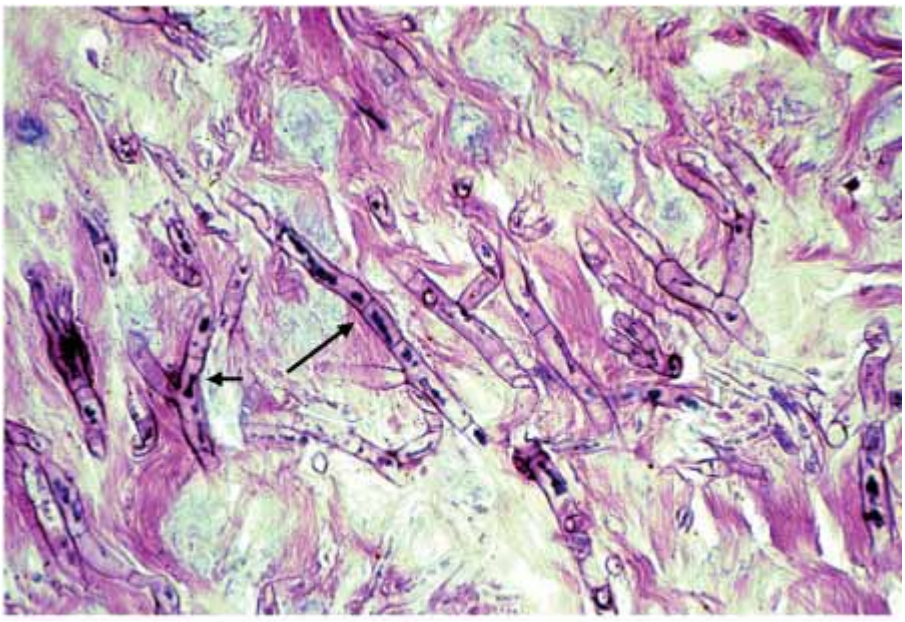
ASPERGILLUS

Disease

- *Aspergillus* species, especially *Aspergillus fumigatus,* cause infections of the skin, eyes, ears, and other organs; "fungus ball" in the lungs; and allergic bronchopulmonary aspergillosis.

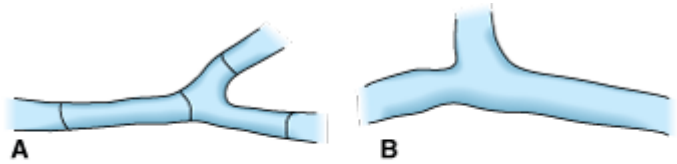
Properties

- *Aspergillus* species exist **only as molds;** they are not dimorphic.
- They have **septate hyphae** that form **V**-shaped (dichotomous) branches.
- The walls are more or less parallel, in contrast to *Mucor* and *Rhizopus* walls, which are irregular.
- The conidia of *Aspergillus* form radiating chains, in contrast to those of *Mucor* and *Rhizopus,* which are enclosed within a sporangium.

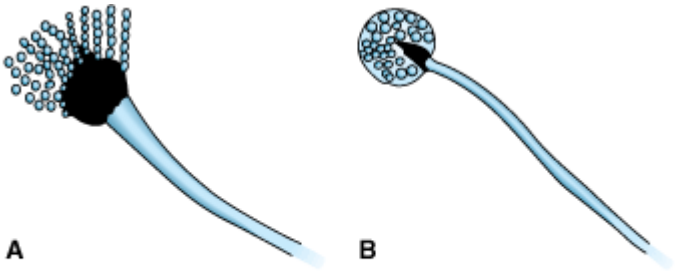


Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: http://www.accessmedicine.com

Aspergillusfumigatus—Septate hyphae. Long arrow points to the septate hyphae of Aspergillus. Note the straight parallel cell walls of this mold. Short arrow points to the typical low-angle, Y-shaped branching. Provider: Professor Henry Sanchez, University of California, San Francisco School of Medicine. With permission.



Aspergillus and *Mucor* in tissue. **A:***Aspergillus* has septate hyphae with **V**-shaped branching. **B:***Mucor* has nonseptate hyphae with right-angle branching.



Aspergillus and *Mucor* in culture. **A:** *Aspergillus* spores form in radiating columns. **B:***Mucor* spores are contained within a sporangium.

Transmission

- These molds are widely distributed in nature.
- They grow on decaying vegetation, producing chains of conidia.
- Transmission is by **airborne conidia.**

Pathogenesis & Clinical Findings

- *A.fumigatus* can colonize and later invade abraded skin, wounds, burns, the cornea, the external ear, or paranasal sinuses.

- It is the most common cause of fungal sinusitis. In immunocompromised persons, especially those with neutropenia, it can invade the lungs and other organs, producing hemoptysis and granulomas.
- Aspergilli are well known for their ability to grow in cavities within the lungs, especially cavities caused by tuberculosis. Within the cavities, they produce an aspergilloma (**fungus ball**), which can be seen on chest x-ray as a radi-opaque structure that changes its position when the patient is moved from an erect to a supine position.
- Allergic bronchopulmonary aspergillosis (ABPA) is an infection of the bronchi by *Aspergillus* species.
- Patients with ABPA have asthmatic symptoms and a high IgE titer against *Aspergillus* antigens, and they expectorate brownish bronchial plugs containing hyphae.
- Asthma caused by the inhalation of airborne conidia, especially in certain occupational settings, also occurs.
- *Aspergillus flavus* growing on cereals or nuts produces aflatoxins that may be carcinogenic or acutely toxic.

Laboratory Diagnosis

- Biopsy specimens show **septate, branching hyphae** invading tissue.
- Cultures show colonies with characteristic radiating chains of conidia.
- However, positive cultures do not prove disease because colonization is common.
- In persons with invasive aspergillosis, there may be high titers of galactomannan antigen in serum.
- Patients with ABPA have high levels of IgE specific for *Aspergillus* antigens and prominent eosinophilia.
- IgG precipitins are also present.

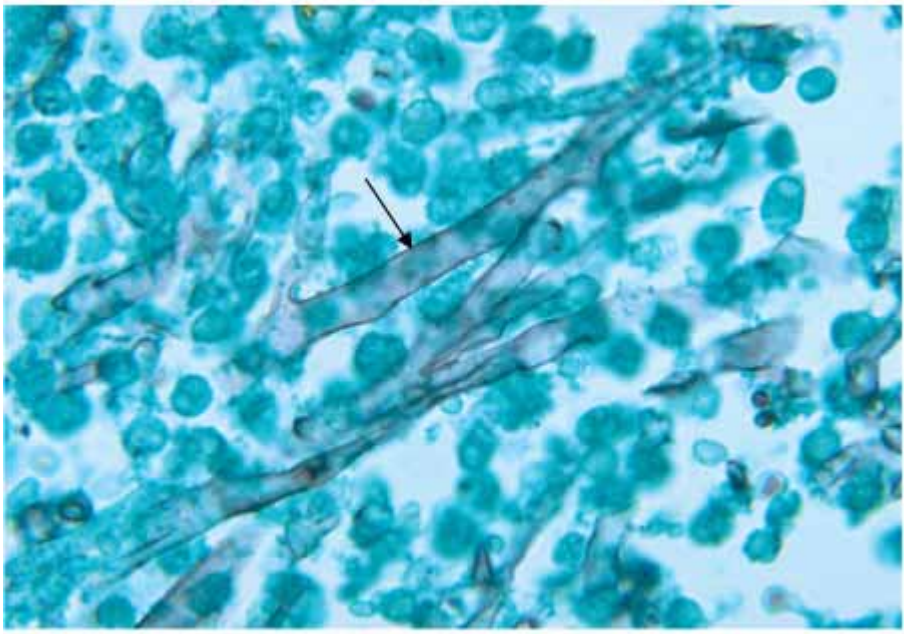
Treatment & Prevention

- Invasive aspergillosis is treated with amphotericin B, but results may be poor.
- Caspofungin may be effective in cases of invasive aspergillosis that do not respond to amphotericin B.
- A fungus ball growing in a sinus or in a pulmonary cavity can be surgically removed.
- Patients with ABPA can be treated with steroids and antifungal agents. There are no specific means of prevention.

MUCOR & RHIZOPUS

- Mucormycosis (zygomycosis, phycomycosis) is a disease caused by saprophytic **molds** (e.g., *Mucor, Rhizopus,* and *Absidia*) found widely in the environment.
- They are not dimorphic. These organisms are transmitted by airborne asexual spores and invade tissues of patients with reduced host defenses.
- They proliferate in the walls of blood vessels, particularly of the paranasal sinuses, lungs, or gut, and cause infarction and necrosis of tissue distal to the blocked vessel.
- Patients with **diabetic ketoacidosis,** burns, or leukemia are particularly susceptible.
- One species, *Rhizopus oryzae,* causes about 60% of cases of mucormycosis.

- In biopsy specimens, organisms are seen microscopically as **nonseptate hyphae** with broad, irregular walls and branches that form more or less at right angles.
- Cultures show colonies with spores contained within a sporangium.
- These organisms are difficult to culture because they are a single, very long cell and damage to any part of the cell can limit its ability to grow.
- If diagnosis is made early, treatment of the underlying disorder, plus administration of amphotericin B and surgical removal of necrotic infected tissue, has resulted in some remissions and cures.



Mucor species—Non-septate hyphae. Arrow points to irregular-shaped, non-septate hyphae of *Mucor*. Provider: CDC/Dr. L. Ajello.

PNEUMOCYSTIS

- *Pneumocystis carinii* is classified as a yeast on the basis of molecular analysis, but medically many still think of it as a protozoan or as an "unclassified" organism.
- It is therefore discussed in Chapter 52 with the blood and tissue protozoa.

- In 2002, taxonomists renamed the human species of *Pneumocystis* as *P. jiroveci* and recommended that *P. carinii* be used only to describe the rat species of *Pneumocystis.*
- There is some controversy surrounding this change of names.

Penicillium marneffei

- *P. marneffei* is a dimorphic fungus that causes tuberculosis-like disease in AIDS patients, particularly in southeast Asian countries such as Thailand.
- It grows as a mold that produces a rose-colored pigment at 25°C but at 37°C grows as a small yeast that resembles *Histoplasma capsulatum.*

- Bamboo rats are the only other known hosts. The diagnosis is made either by growing the organism in culture or by using fluorescent-antibody staining of affected tissue.
- The treatment of choice consists of amphotericin B for 2 weeks followed by oral itraconazole for 10 weeks.
- Relapses can be prevented with prolonged administration of oral itraconazole.

Pseudallescheria boydii

- *P. boydii* is a mold that causes disease primarily in immunocompromised patients.
- The clinical findings and the microscopic appearance of the septate hyphae in tissue closely resemble those of *Aspergillus.*
- In culture, the appearance of the conidia (pear-shaped) and the color of the mycelium (brownish-gray) of *P. boydii* are different from those of *Aspergillus.*
- The drug of choice is either ketoconazole or itraconazole because the response to amphotericin B is poor.
- Debridement of necrotic tissue is important as well.

Fusarium solani

- *F. solani* is a mold that causes disease primarily in neutropenic patients.
- Fever and skin lesions are the most common clinical features.
- The organism is similar to *Aspergillus* in that it is a mold with septate hyphae that tends to invade blood vessels.
- Blood cultures are often positive in disseminated disease.
- In culture, banana-shaped conidia are seen.
- Liposomal amphotericin B is the drug of choice. Indwelling catheters should be removed or replaced.
- In 2006, an outbreak of *Fusarium* keratitis (infection of the cornea) occurred in people who used a certain contact lens solution.