Med. Microbiology 1

lec2:FUNDAMENTALS OF MICROBIOLOGY

Presented by:

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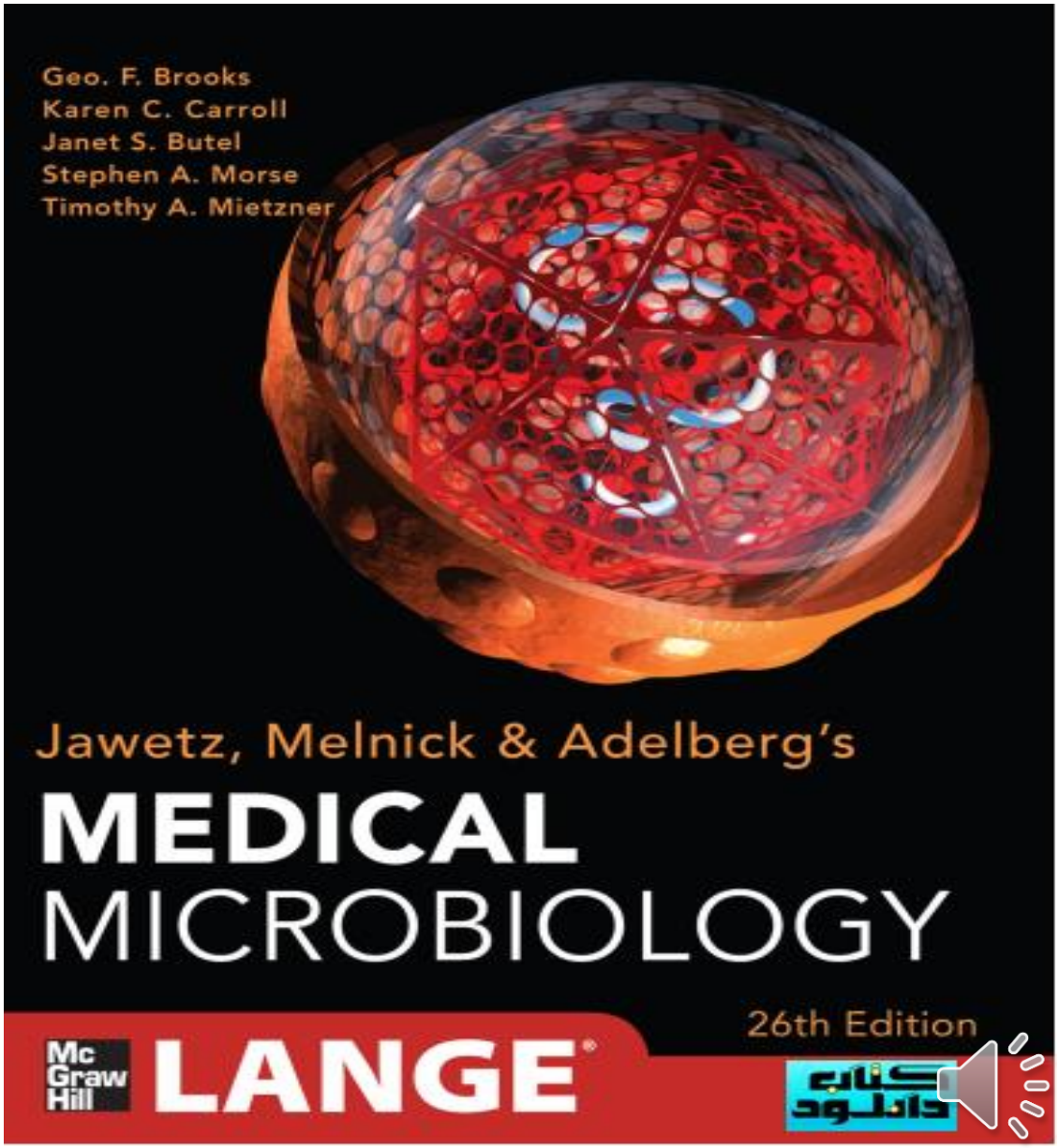
Reference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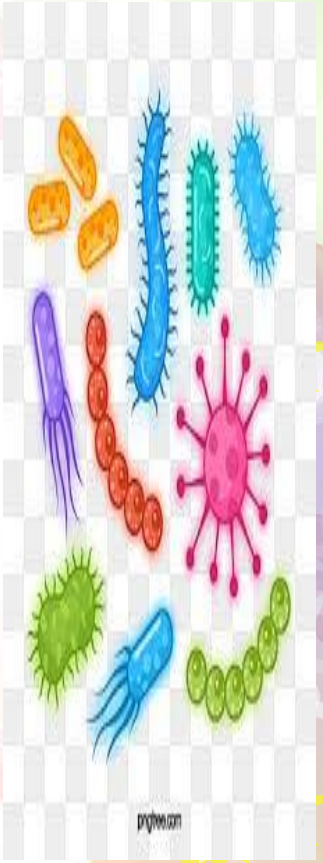
Science of Microbiology

- **M**icrobiology is the study of microorganisms, a large and diverse group of microscopic organisms that exist as single cells or cell clusters; it also includes viruses, which are microscopic but not cellular.
- **Microorganisms have a tremendous impact on all life and the physical and chemical makeup of our planet. They are responsible for cycling the chemical elements essential for life, including carbon, nitrogen, sulfur, hydrogen, and oxygen; more photosynthesis is carried out by microorganisms than by green plants.**

TAXONOMY—THE VOCABULARY OFMEDICALMICROBIOLOGY



Classification of bacteria requires experimental and observational techniques; this is because biochemical, physiologic, genetic, and morphologic properties are often necessary for an adequate description of a taxon.
Nomenclature refers to the naming of an organism by international rules (established by a recognized group of medical professionals) according to its characteristics.

Taxonomic ranks form the basis for the organization of bacteria. Linnaean taxonomy is the system most familiar to biologists. It uses the formal taxonomic ranks of kingdom, phylum, class, order, family, genus, and species. The lower ranks are approved by a consensus of experts in the scientific community (Table 1). Of these ranks, the family, genus, and species are the most useful

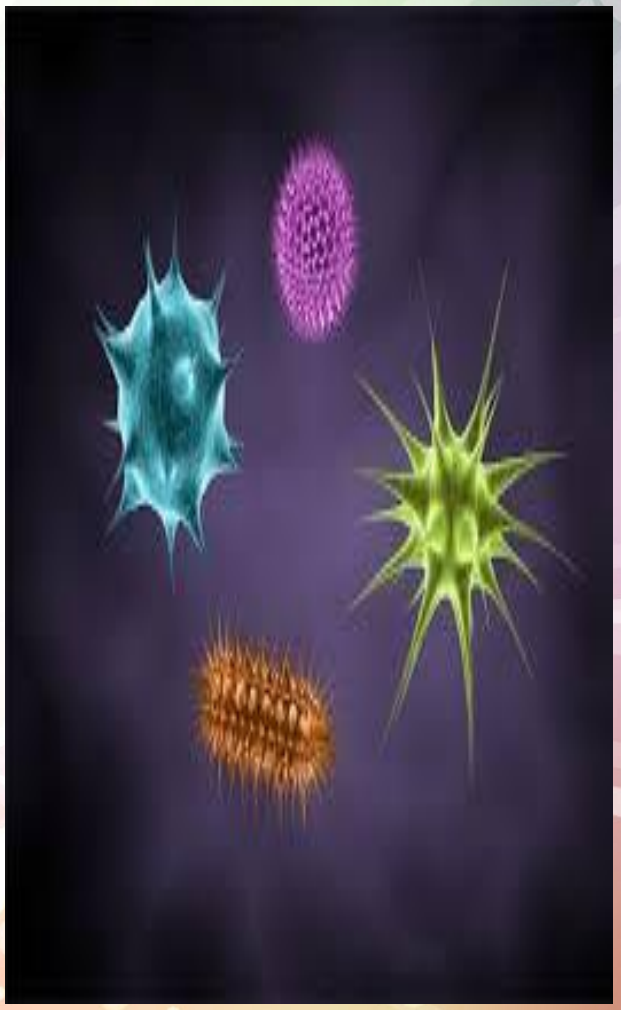


Table: 1 Taxonomic ranks

Formal rank	**example**
Kingdom	Prokaryotae
Division	Gracilicutes
Class	Scotobacteria
Order	Eubacteriales
Family	Enterobacteriaceae
Genus	*Escherichia*
Species	*coli*
Subtype	*Escherichia coli* O157: H7

Criteria For Classification Of Bacteria Growth on Media

- The general cultivation of most bacteria requires media rich in metabolic nutrients. These media generally include agar, a carbon source, and an acid hydrolysate or enzymatically degraded source of biologic material (eg, casein). Because of the undefined composition of the latter, these types of media are referred to as complex media. Clinical samples from normally nonsterile sites (eg, the throat or the colon) contain multiple species of organisms, including potential pathogens and resident microbial flora. Media can be **nonselective** or **selective**;

Solid

Liquid

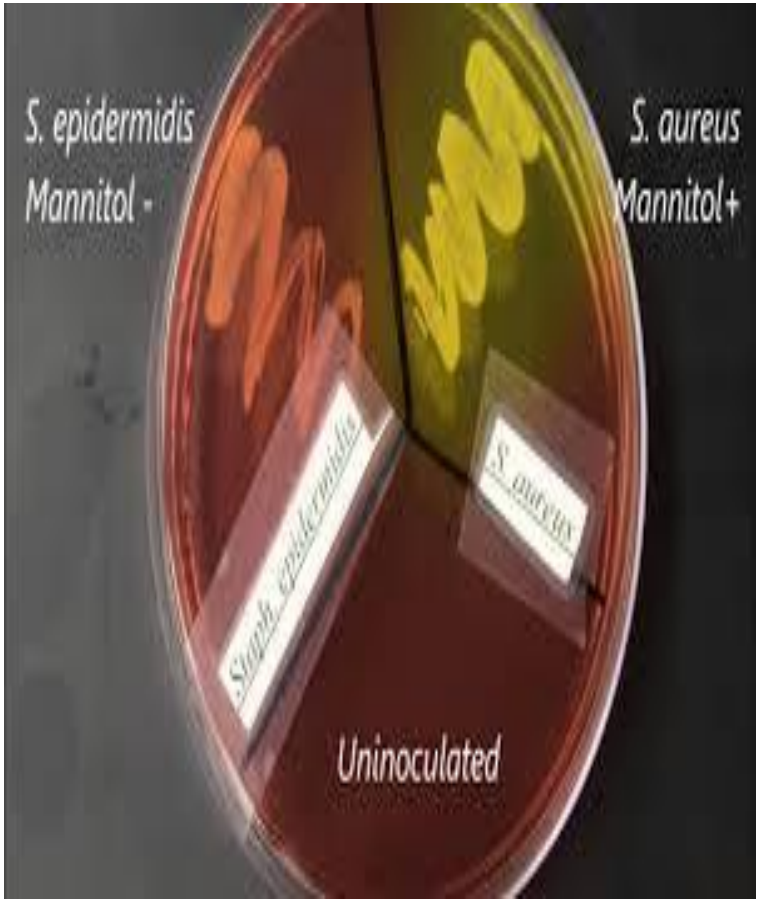
semi-solid

Culture Media

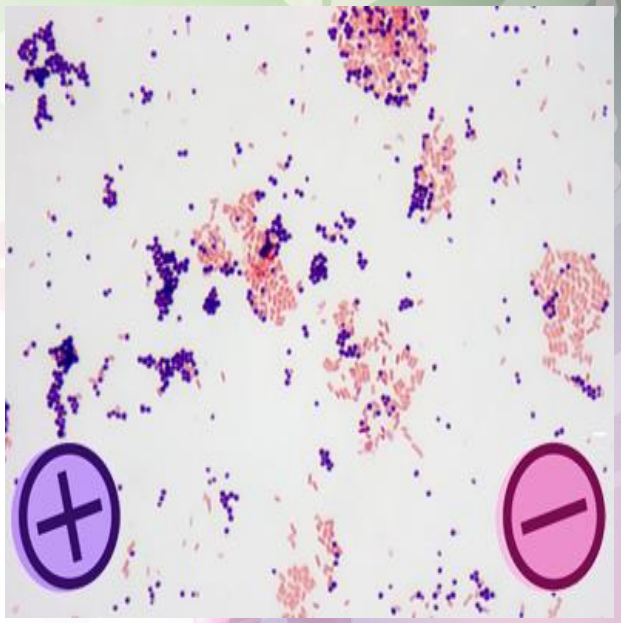
- **Nonselective Media**

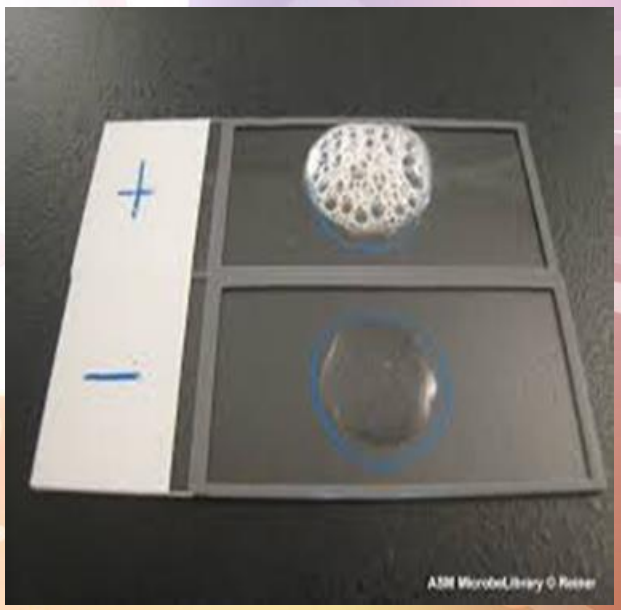
Blood agar and chocolate agar are examples of complex, nonselective media, which support the growth of many different bacteria. These media are intended to cultivate as many species as possible, thus giving rise to numerous types of bacterial colonies.

- **Selective Media**
- selective media are used to eliminate (or reduce) the large numbers of irrelevant bacteria in these specimens. The basis for selective media is the incorporation of an inhibitory agent that specifically selects against the growth of irrelevant bacteria. Examples of such agents are:
- • Sodium azide—selects for gram-positive bacteria over gram-negative bacteria
- • Bile salts (sodium deoxycholate)—select for gram-negative enteric bacteria and inhibit gram-negative mucosal and most gram-positive bacteria.



- **. Differential Media**
- Upon culture, some bacteria produce characteristic pigments, and others can be differentiated on the basis of their complement of extracellular enzymes; the activity of these enzymes often can be detected as zones of clearing surrounding colonies grown in the presence of insoluble substrates (eg, zones of hemolysis in agar medium containing red blood cells).





- **Bacterial Microscopy (**Gram stain)

This staining technique broadly divides bacteria on the basis of fundamental differences in the structure of their cell walls.

Biochemical Tests

the catalase activity can be used, for example, to differentiate between the G+ cocci; the species staphylococci are catalase +

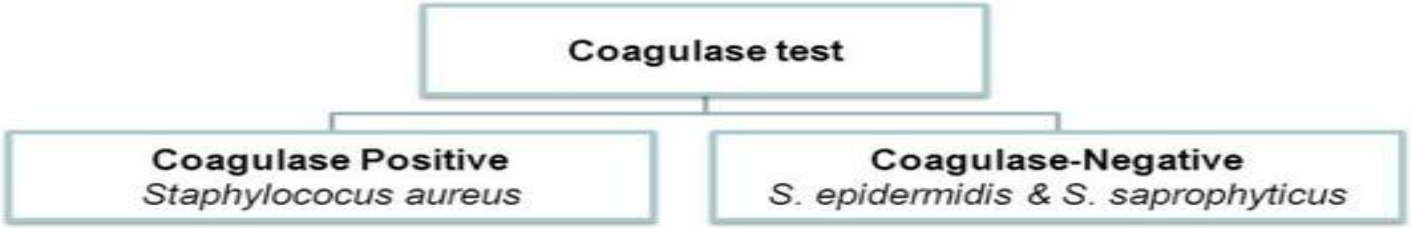
streptococci are catalase _.

If the organism is demonstrated to be catalase positive (Staphylococcus spp.), the species can be subdivided by a coagulase test into *Staph. aureus* (coagulase +) or *Staph. epidermitidis* (coagulase _)

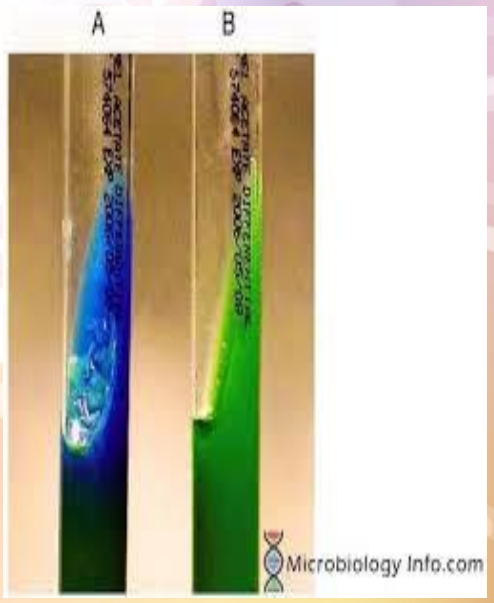
Coagulase Test

Principle:

- This test is used to differentiate between *S. aureus* (CPS) & other *Staphylococcus* species (CNS)
- This test is done by tube method or slide method

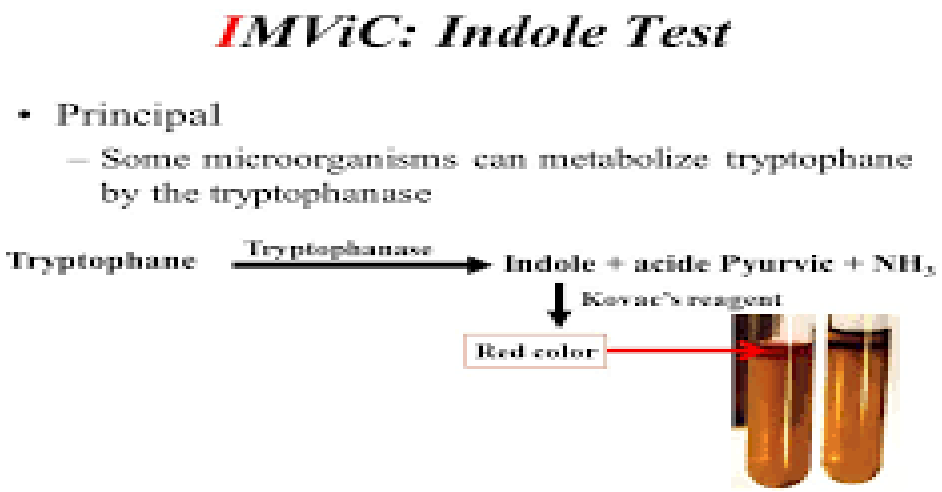


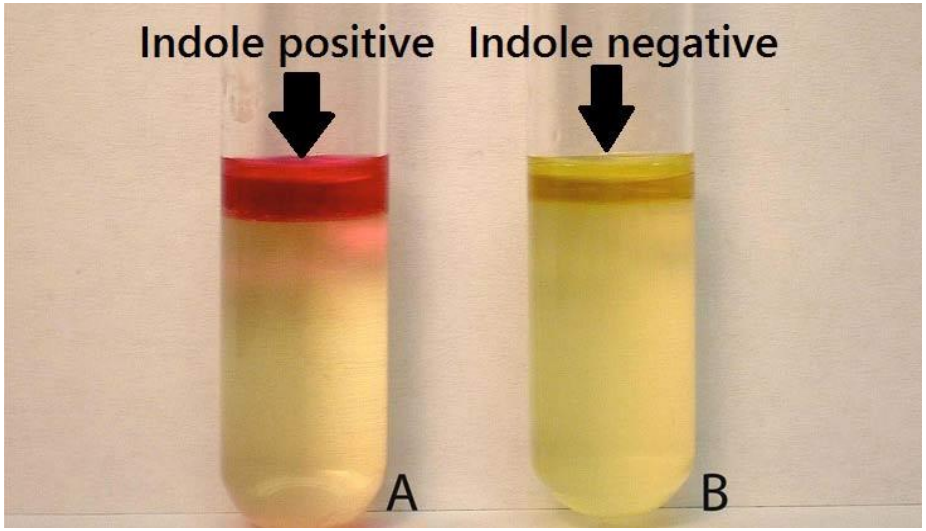
Biochemical Tests used to Differentiate Among Bacteria



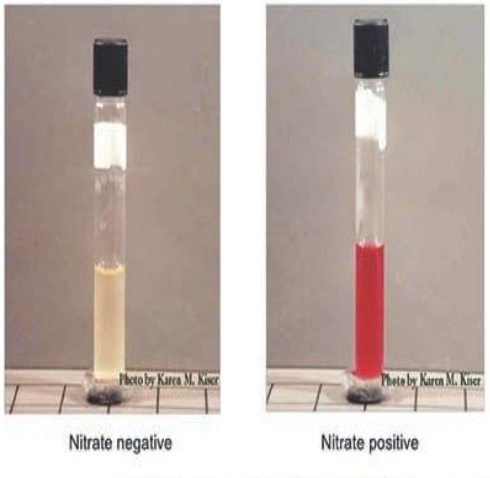
- **Carbohydrate breakdown:** carbohydrates (eg, glucose, sucrose, and lactose) has been applied to the identification of most groups of bacteria.
- have proved useful for taxonomic purposes.
- **Catalase production**
- **Citrate utilization.** An agar medium that contains sodium citrate as the sole carbon source
- Bacteria that grow on this medium are termed citrate-positive.
- **Coagulase**.

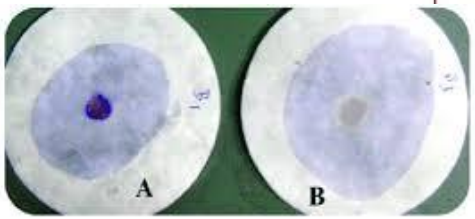
- **Decarboxylases and deaminases**
- **Hydrogen sulfide**
- **Indole**





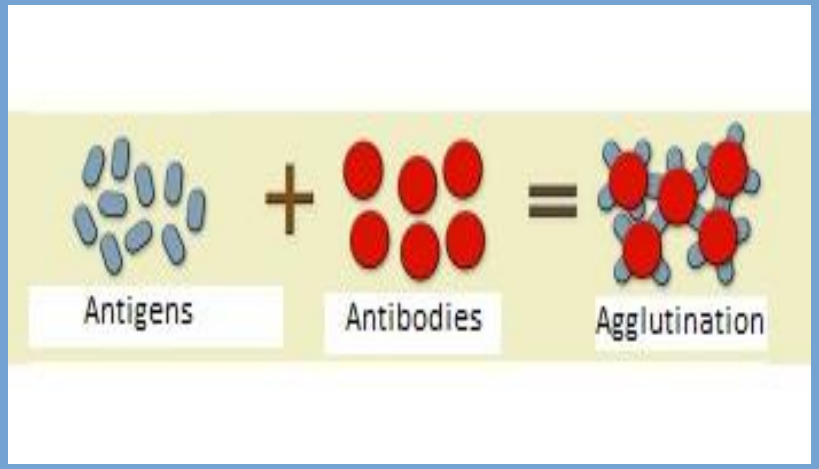
- Nitrate reduction
- Oxidase production
- Urease production
- Voges–Proskauer test





Immunologic Tests—Serotypes, Serogroups, and Serovars

- The designation “sero” simply indicates the use of antibodies that react with specific bacterial cell surface structures such as lipopolysaccharide (LPS), flagella, or capsular antigens. The terms “serotype,” serogroups,” and “serovars” are, for all practical purposes, identical—they all use the specificity of these antibodies to subdivide strains of a particular bacterial species.



Genetic Instability

- Developments in molecular biology now make it possible to investigate the relatedness of genes or genomes by comparing sequences among different bacteria. For these cases, genetic instability can cause some traits to be highly variable within a biologic group or even within a specific taxonomic group. For example, antibiotic resistance genes or genes encoding enzymes (eg, lactose utilization) may be carried on **plasmids** or **bacteriophages**, **extra-chromosomal** genetic elements that may be transferred among unrelated bacteria or that may be lost from a subset of bacterial strains identical in all other respects.

Description of The Major Categories and Groups of Bacteria

Bergey's Manual of Systematic Bacteriology

- The definitive work on the taxonomic organization of bacteria is the latest edition of ***Bergey's Manual of Systematic Bacteriology***. First published in 1923, this publication taxonomically classifies, in the form of a key, known bacteria that have or have not been cultured or well-described. A companion volume, Bergey's Manual of Determinative Bacteriology, serves as an aid in the identification of bacteria that have been described and cultured. The major bacteria that cause infectious diseases, as categorized in Bergey's Manual, are listed in Table 2.

- There are two different groups of **prokaryotic organisms**, eubacteria and archaebacteria.
- Both are small unicellular organisms that replicate asexually. Eubacteria refer to classic bacteria as science has historically understood them.
- They lack a true nucleus, have characteristic lipids that make up their membranes, possess a peptidoglycan cell wall, and have a protein and nucleic acid synthesis machinery that can be selectively inhibited by antimicrobial agents.
- In contrast, archaebacteria do not have a classic peptidoglycan cell wall and have many characteristics (eg, protein synthesis and nucleic acid replication machinery) that are similar to those of eukaryotic cells

Major Categories and Groups of Bacteria That Cause Disease in Humans as Part of an Identification Scheme Described in *Bergey's Manual of Determinative Bacteriology*, 9th ed.

Bergey's Manual of Systematic Bacteriology	
I. Gram-negative eubacteria that have cell walls	
Group 1: The spirochetes	*Treponema*
	Borrelia
	Leptospira
Group 2: Aerobic/microaerophilic, motile helical/vibroid gram-negative bacteria	*Campylobacter*
	Helicobacter
	Spirillum
Group 3: Nonmotile (or rarely motile) curved bacteria	None
Group 4: Gram-negative aerobic/microaerophilic rods and cocci	*Alcaligenes*
	Bordetella
	Brucella
	Francisella
	Legionella
	Moraxella
	Neisseria
	Pseudomonas
	Rochalimaea
	Bacteroides (some species)
Group 5: Facultatively anaerobic gram-negative rods	*Escherichia* (and related coliform bacteria)
	Klebsiella
	Proteus
	Providencia
	Salmonella
	Shigella
	Yersinia
	Vibrio
	Haemophilus
	Pasteurella
Group 6: Gram-negative, anaerobic, straight, curved, and helical rods	*Bacteroides*
	Fusobacterium
	Prevotella
Group 7: Dissimilatory sulfate- or sulfur-reducing bacteria	None
Group 8: Anaerobic gram-negative cocci	None
Group 9: The rickettsiae and chlamydiae	*Rickettsia*
	Coxiella
	Chlamydia
Group 10: Anoxygenic phototrophic bacteria	None
Group 11: Oxygenic phototrophic bacteria	None
Group 12: Aerobic chemolithotrophic bacteria and assorted organisms	None
Group 13: Budding or appendaged bacteria	None
Group 14: Sheathed bacteria	None
Group 15: Nonphotosynthetic, nonfruiting gliding bacteria	*Capnocytophaga*
Group 16: Fruiting gliding bacteria: the myxobacteria	None
II. Gram-positive bacteria that have cell walls	
Group 17: Gram-positive cocci	*Enterococcus*
	Peptostreptococcus
	Staphylococcus
	Streptococcus
Group 18: Endospore-forming gram-positive rods and cocci	*Bacillus*
	Clostridium
Group 19: Regular, nonsporing gram-positive rods	*Erysipelothrix*
	Listeria
Group 20: Irregular, nonsporing gram-positive rods	*Actinomyces*
	Corynebacterium
	Mobiluncus
Group 21: The mycobacteria	*Mycobacterium*
Groups 22–29: Actinomycetes	*Nocardia*
	Streptomyces
	Rhodococcus
III. Cell wall-less eubacteria: The mycoplasmas or mollicutes	
Group 30: Mycoplasmas	*Mycoplasma*
	Ureaplasma
IV. Archaebacteria	
Group 31: The methanogens	None
Group 32: Archaeal sulfate reducers	None
Group 33: Extremely halophilic archaebacteria	None
Group 34: Cell wall-less archaebacteria	None
Group 35: Extremely thermophilic and hyperthermophilic sulfur metabolizers	None

Pathogenesis of Bacterial Infection

- The pathogenesis of bacterial infection includes initiation of the infectious process and the mechanisms that lead to the development of signs and symptoms of disease. The biochemical, structural, and genetic factors that play important roles in bacterial pathogenesis.
- Characteristics of bacteria that are pathogens include transmissibility, adherence to host cells, persistence, invasion of host cells and tissues, toxigenicity, and the ability to evade or survive the host's immune system.
- Resistance to antimicrobials and disinfectants can also contribute to virulence, or an organism's capacity to cause disease.
- Many infections caused by bacteria that are commonly considered to be pathogens are in apparent or asymptomatic
- Disease occurs if the bacteria or immunologic reactions to their presence cause sufficient harm to the person.

Terms frequently used in describing aspects of pathogenesis are defined in the Glossary

- **Adherence (adhesion, attachment**The process by which bacteria stick to the surfaces of host cells.
- **Carrier:** A person or animal with asymptomatic infection that can be transmitted to another susceptible person or animal.

Infection: Multiplication of an infectious agent within the body.

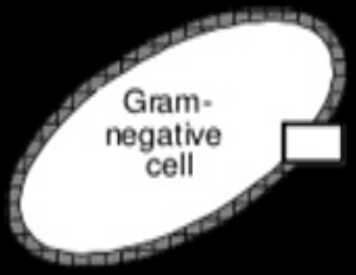
- **Invasion:** The process whereby bacteria, animal parasites, fungi, and viruses enter host cells or tissues and spread in the body.
- **Microbiota:** Microbial flora harbored by normal, healthy individuals.
- **Non-pathogen:** A microorganism that does not cause disease; may be part of the normal microbiota.

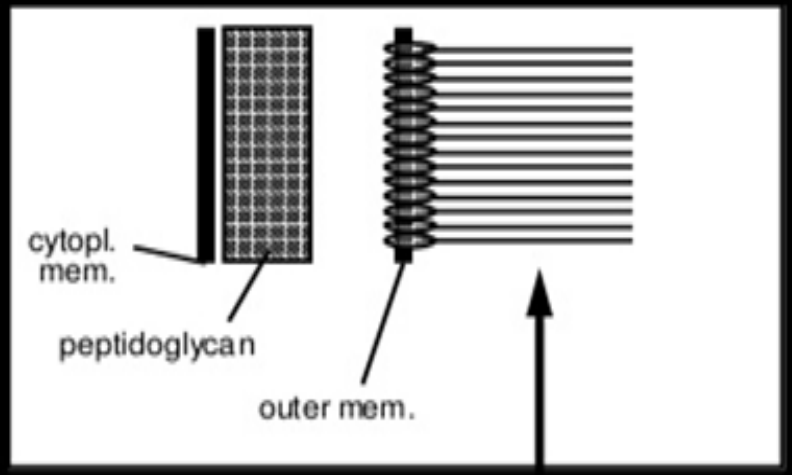
- **Opportunistic pathogen:** An agent capable of causing disease only when the host's resistance is impaired (i.e., when the patient is"immunocompromised").
- **Pathogen:** A microorganism capable of causing disease.
- **Pathogenicity:** The ability of an infectious agent to cause disease. (See also virulence.)
- **Superantigens:** Protein toxins that activate the immune system by binding to major histocompatibility complex (MHC) molecules and T-cell receptors (TCR) and stimulate large numbers of T cells to produce massive quantities of cytokines.
- **Toxigenicity:** The ability of a microorganism to produce a toxin that contributes to the development of disease.
- **Virulence:** The quantitative ability of an agent to cause disease. Virulent agents cause disease when introduced into the host in small numbers. Virulence involves adherence, persistence, invasion, and toxigenicity (see above).

Toxins

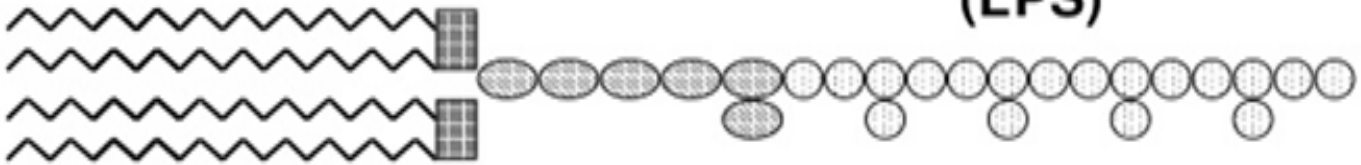
Toxins produced by bacteria are generally classified into two groups: exotoxins and endotoxins. Exotoxins are proteins that are most often excreted from the cell. However some exotoxins accumulate inside the cell and are either injected directly into the host or are released by cell lysis. Endotoxins are lipid molecules that are components of the bacterial cell membrane.

Endotoxin of Gram-negatives



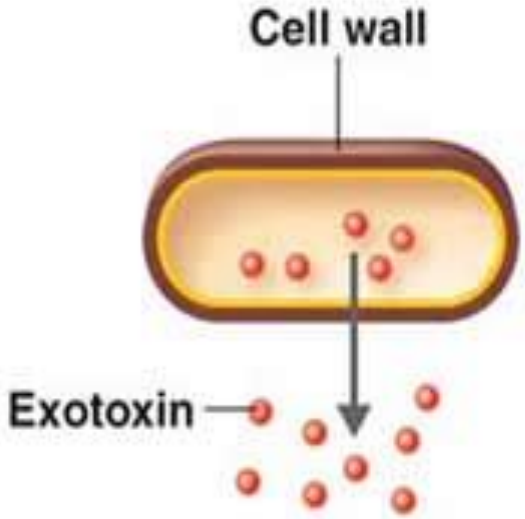


Lipopolysaccharide (LPS)

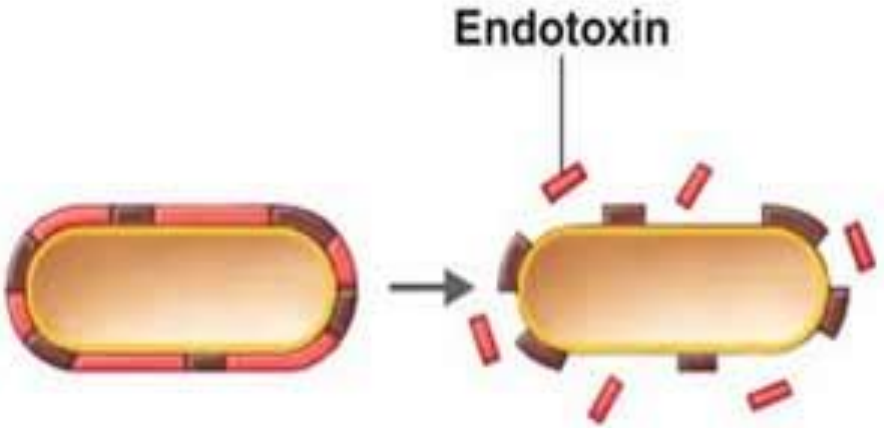


Lipid A	Core polysaccharide	O sidechain
The toxic part	Helps solubilise Lipid A	Somatic antigen

Differences Between Exotoxins and Endotoxins



(a) Exotoxins are proteins produced inside pathogenic bacteria, most commonly gram-positive bacteria, as part of their growth and metabolism. The exotoxins are then secreted or released into the surrounding medium following lysis.



(b) Endotoxins are the lipid portions of lipopolysaccharides (LPSs) that are part of the outer membrane of the cell wall of gram-negative bacteria (lipid A; see Figure 4.13c). The endotoxins are liberated when the bacteria die and the cell wall breaks apart.

The Role of Bacterial Biofilms

- A biofilm is an aggregate of interactive bacteria attached to a solid surface or to each other and encased in an exo-polysaccharide matrix. This is distinct from planktonic or free-living bacteria, in which interactions of the microorganisms do not occur in the same way. Biofilms form a slimy coat on solid surfaces and occur throughout nature. A single species of bacteria may be involved or more than one species may coaggregate to form a biofilm. Fungi, including yeasts, are occasionally involved. After a biofilm is formed, quorum-sensing molecules produced by the bacteria in the biofilm accumulate, resulting in a modification of the metabolic activity of the bacteria.

- The bacteria in the exopolysaccharide matrix may be protected from the host's immune mechanisms. This matrix also functions as a diffusion barrier for some antimicrobials, but other antimicrobials may bind to it. **Some of the bacteria within the biofilm show marked resistance to antimicrobials compared with the same strain of bacteria grown free living in broth, which helps to explain why it is so difficult to treat infections associated with biofilms.** Biofilms are important in human infections that are persistent and difficult to treat.
- A few examples include *Staphylococcus epidermidis* and *S aureus* infections of central venous catheters, eye infections such as that occur with contact lenses and intraocular lenses, in dental plaque, and in prosthetic joint infections.

5 Stages of Biofilm Development

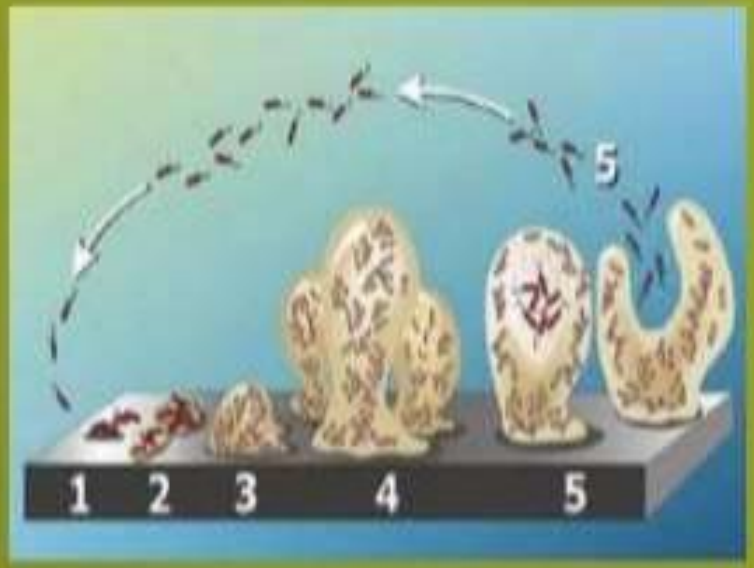
Stage 1:
- Initial reversible attachment of planktonic microbes to a surface. Mostly physical.

Stage 2
- Permanent chemical attachment.
- Single layer slime development. (EPS)

Stage 3
- Early vertical development.
- Start of 3D structure formation.

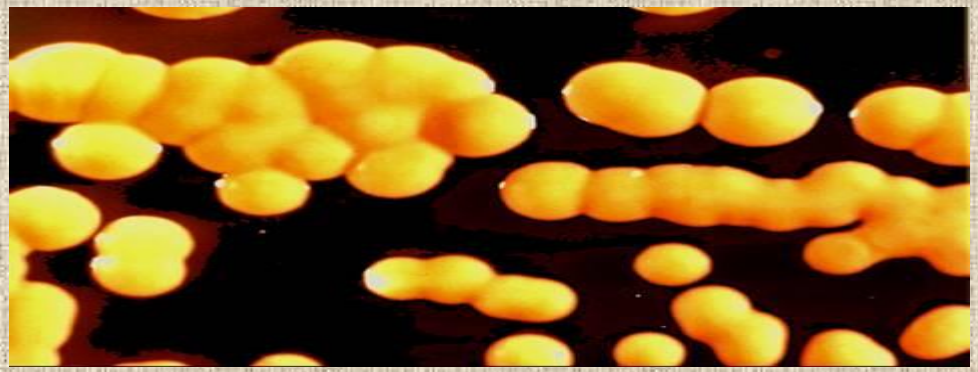
Stage 4
- Multiple towers are formed with channels linking them. (nutrient movement)

Stage 5
- Mature biofilm formed.
- Dispersal of more planktonic microbes.



- Thanks for attention
- Any Question?

Physiology of Bacteria. Growth and reproduction of Bacteria



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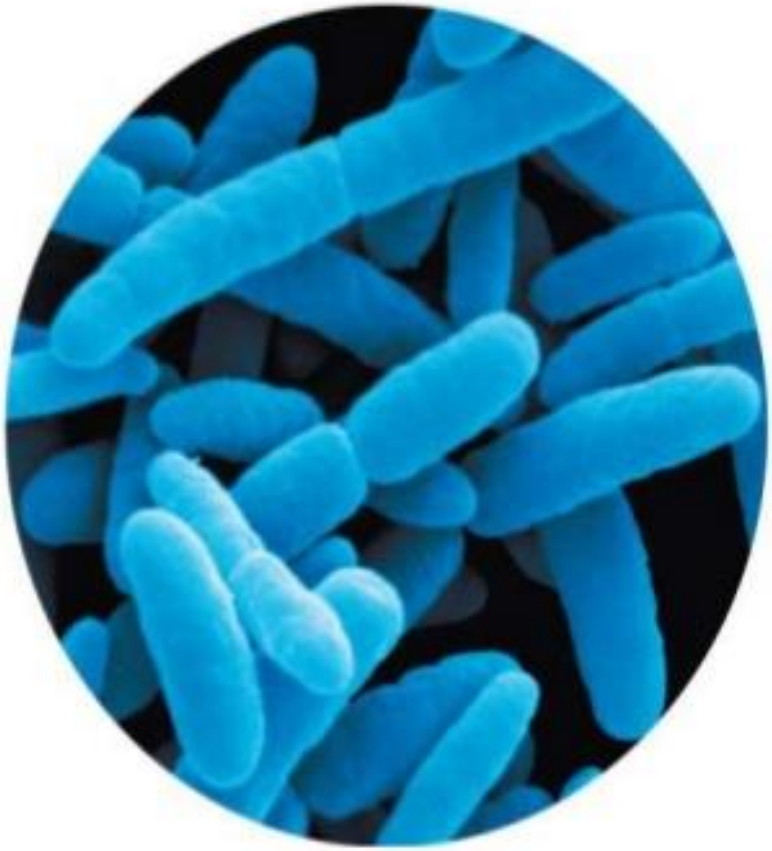
WHAT IS PHYSIOLOGY ?

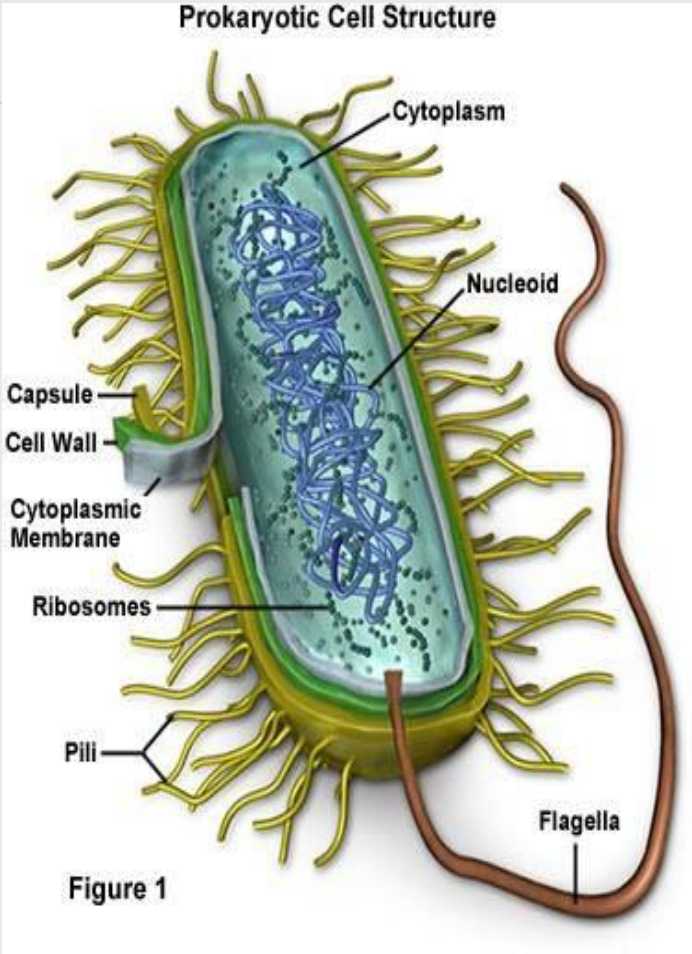
IT'S THE

GROWTH, NU TRITION AND METABOLISM



Metabolism refers to all the biochemical reactions that occur in a cell or organism.

The study of bacterial metabolism focuses on the chemical diversity of substrate **oxidations and dissimilation reactions** (reactions by which substrate molecules are broken down), which normally function in bacteria to **generate energy.**



- **Metabolism** is the process of building up chemical compounds in the cell and their breaking down during activity to receive the required energy and the building elements.
- **Metabolism** comprises
- of ***anabolism*** (assimilation) and ***catabolism*** (dissimilation)

Classification

- Based on Nutrition bacteria are classified as :

1. Autotrophs – can synthesise all their organic compounds by utilising atmospheric CO_2 & N_2. No medical importance.
2. Heterotrophs – unable to synthesise their own metabolites & depend on preformed organic compounds. All pathogens

- ***Autotrophs*** are free-living, most of which can use carbon dioxide as their carbon source. The energy can be obtained from:

- sunlight – **protoautotrophs** (get energy from photochemical reactions)
- inorganic compounds, by oxidation – **chemoautotrophs** (get energy from chemical reactions)
- ***Heterotrophs*** are generally parasitic bacteria, requiring more complex organic compounds than carbon dioxide, e.g. sugars, as their source of carbon and energy.

- **Growth Factors**

  Organic compounds required in minute quantities and are not synthesized by bacteria, also called **bacterial vitamins.**

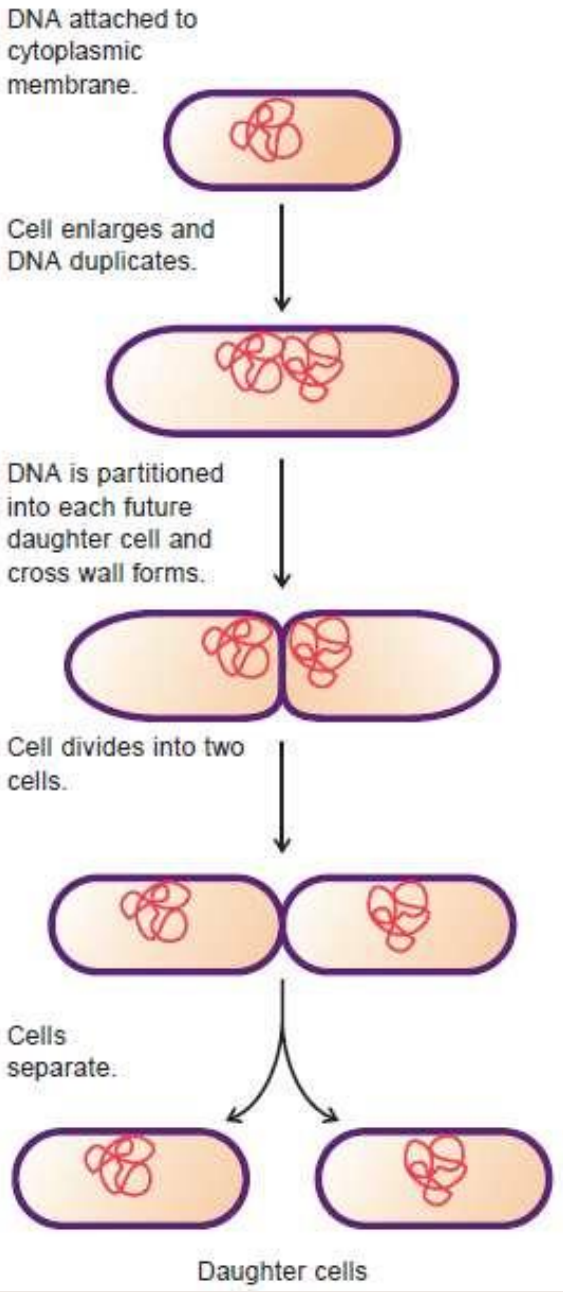
  Such as amino acids, vitamins, purines, and pyrimidines

  E.g.…Neisseria spp require at least 40 additional ingredients, including 7 vitamins and all of the 20 amino acids.

Bacterial Growth

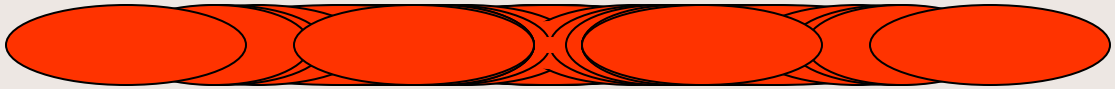
- It is an increase in number of population rather than in size.
  Bacteria divide by binary fission.

Binary fission



BINARY FISSION

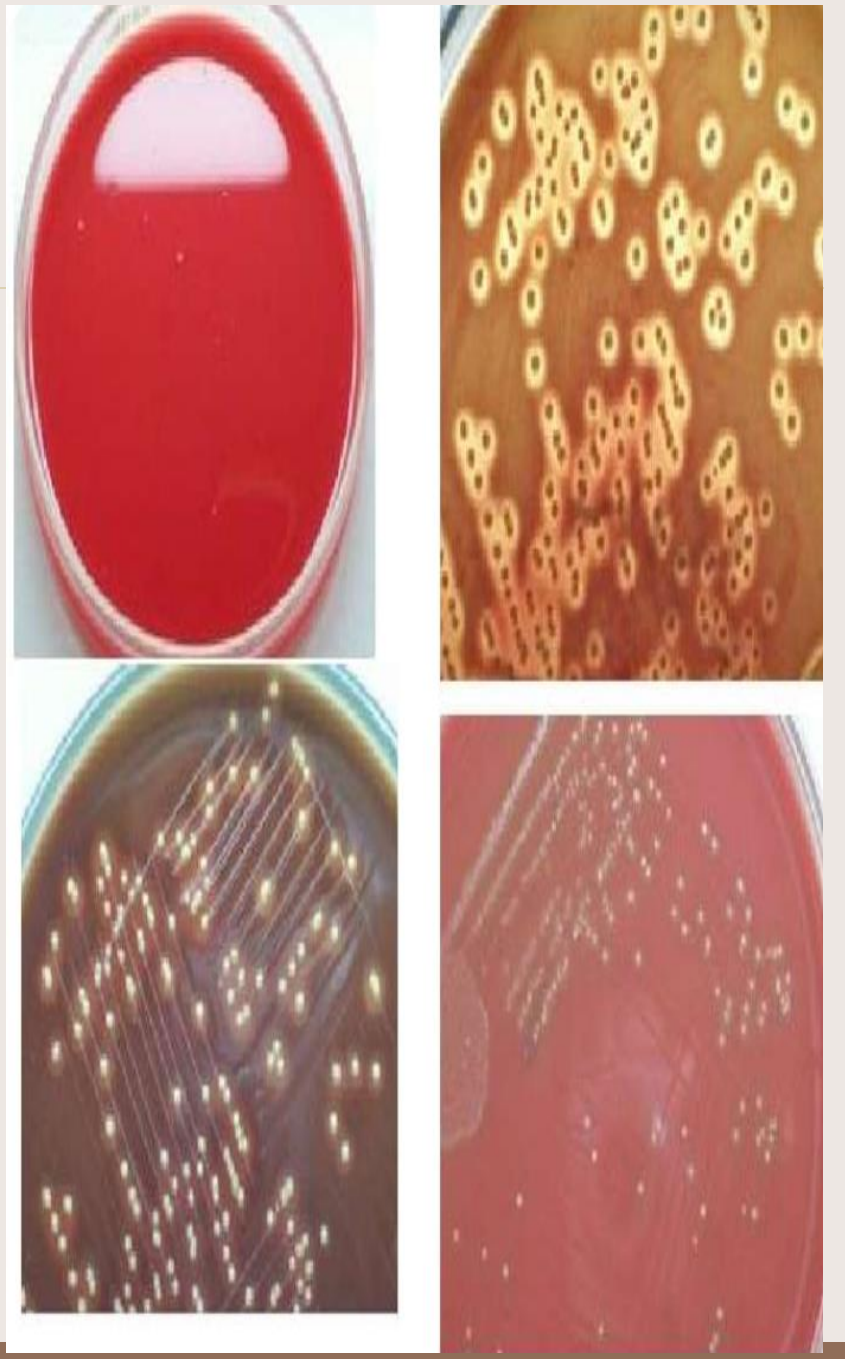
- division exactly in half
- most common means of bacterial reproduction
  - forming two equal size progeny
  - genetically identical offspring
  - cells divide in a geometric progression doubling cell number



Generation time

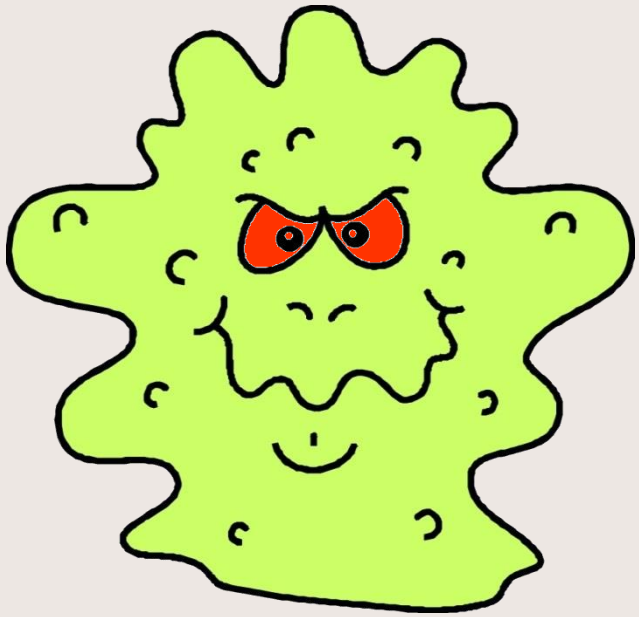
- Time required for abacterium to give rise to 2 daughter cells under optimum conditions .Also called population doubling time.
- *Escherichia coli* – 20 mins.
- *Mycobacterium tuberculosis* – 20 hrs.
- *M.leprae* – 20 days.

- **colony – formed by bacteria growing on solid media.(20-30 cell divisions)**
  Each bacterial colony represents a clone of cells derived from a single parent cell.



TEMPERATURE

- One of the most important factors
- optimal growth temperature
  - temperature range at which the highest rate of reproduction occurs
- optimal growth temperature for human pathogens ????



TEMPERATURE

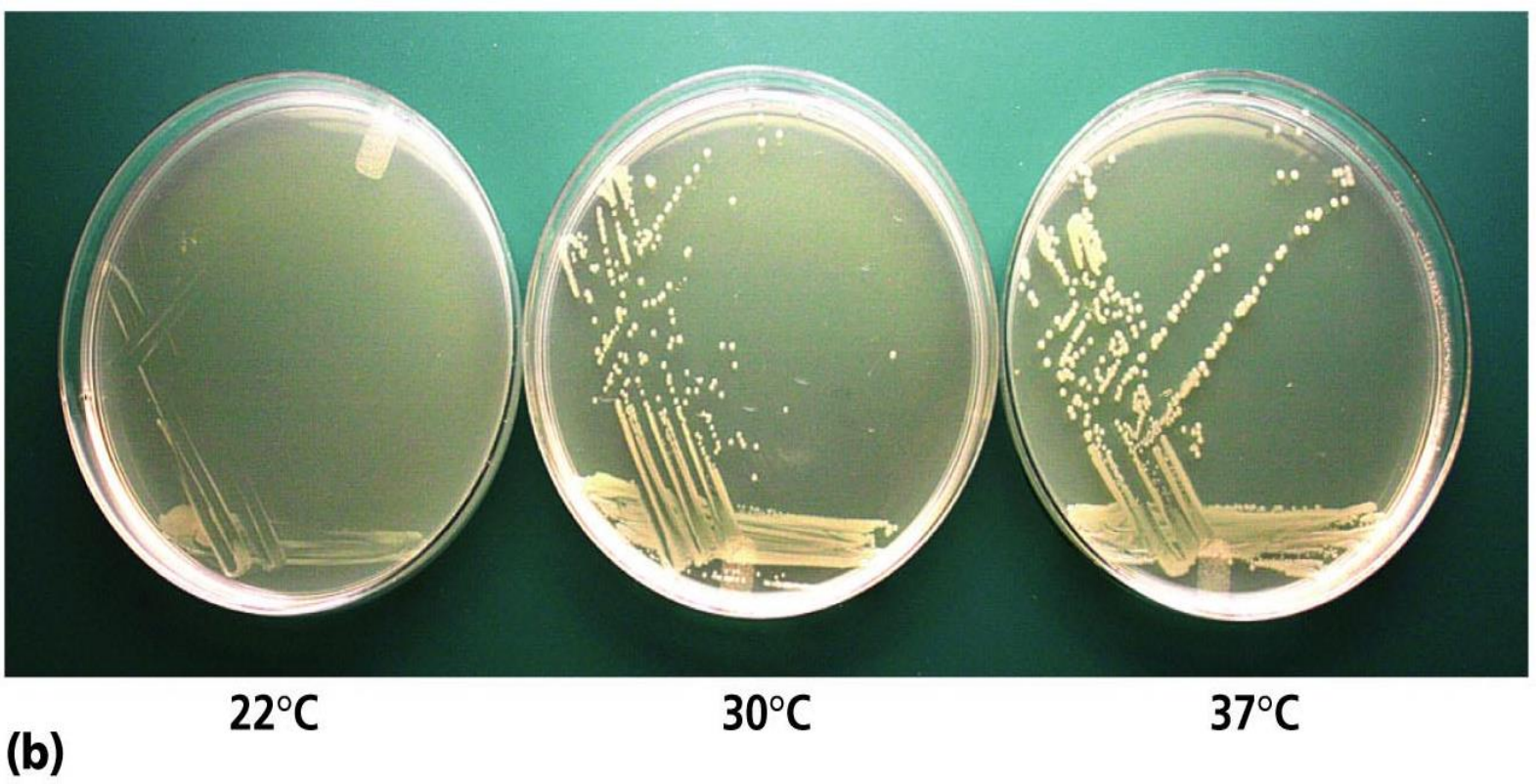
- Microorganisms can be categorized based on their optimal temperature requirements

  - Psychrophiles
    - 0 - 20 ºC
    - Produce enzymes that functions optimally in cold.

  - Mesophiles
    - 20 - 40 ºC
    - Are mostly pathogenic bacteria.
      Escherichia coli , staphylococcus aureus , etc.

  – Thermophiles
    - 40 - 90 ºC
    - Contain heat stable enzymes and proteins.

Effects of Temperature on Growth



Respiration in Bacteria

Obligate Aerobe

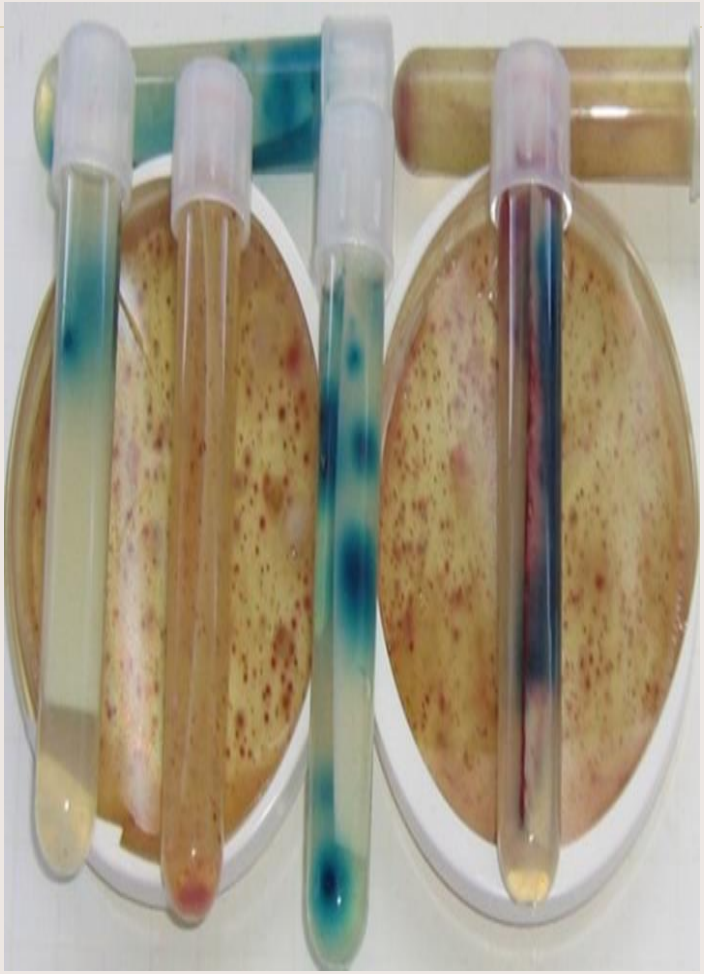
Microaerophile

Obligate Anaerobe

Facultative Anaerobe (Facultative Aerobe)

Aerotolerant Anaerobe

Capneic bacteria



Categories of Oxygen Requirement

Aerobe – utilizes oxygen and can detoxify it

- **Obligate aerobe -** cannot grow without oxygen (*Mycobacterium tuberculosis*, *Micrococcus spp., Bacillus spp., Pseudomonas spp.*
- **Facultative anaerobe** – utilizes oxygen but can also grow in its absence (*Echericihia* spp., *Salmonella* spp., *Staphylococcus* spp.)
- **Microaerophylic** – requires only a small amount of oxygen (*Helycobacter spp., Lactobacillus* spp.)

Categories of Oxygen Requirement

Anaerobe – does not utilize oxygen

- **obligate anaerobe -** lacks the enzymes to detoxify oxygen so cannot survive in an oxygen environment (*Clostridium spp., Bacteroides spp.)*
- **aerotolerance anaerobes** – do no utilize oxygen but can survive and grow in its presence (*Streptococcus pyogenes*)

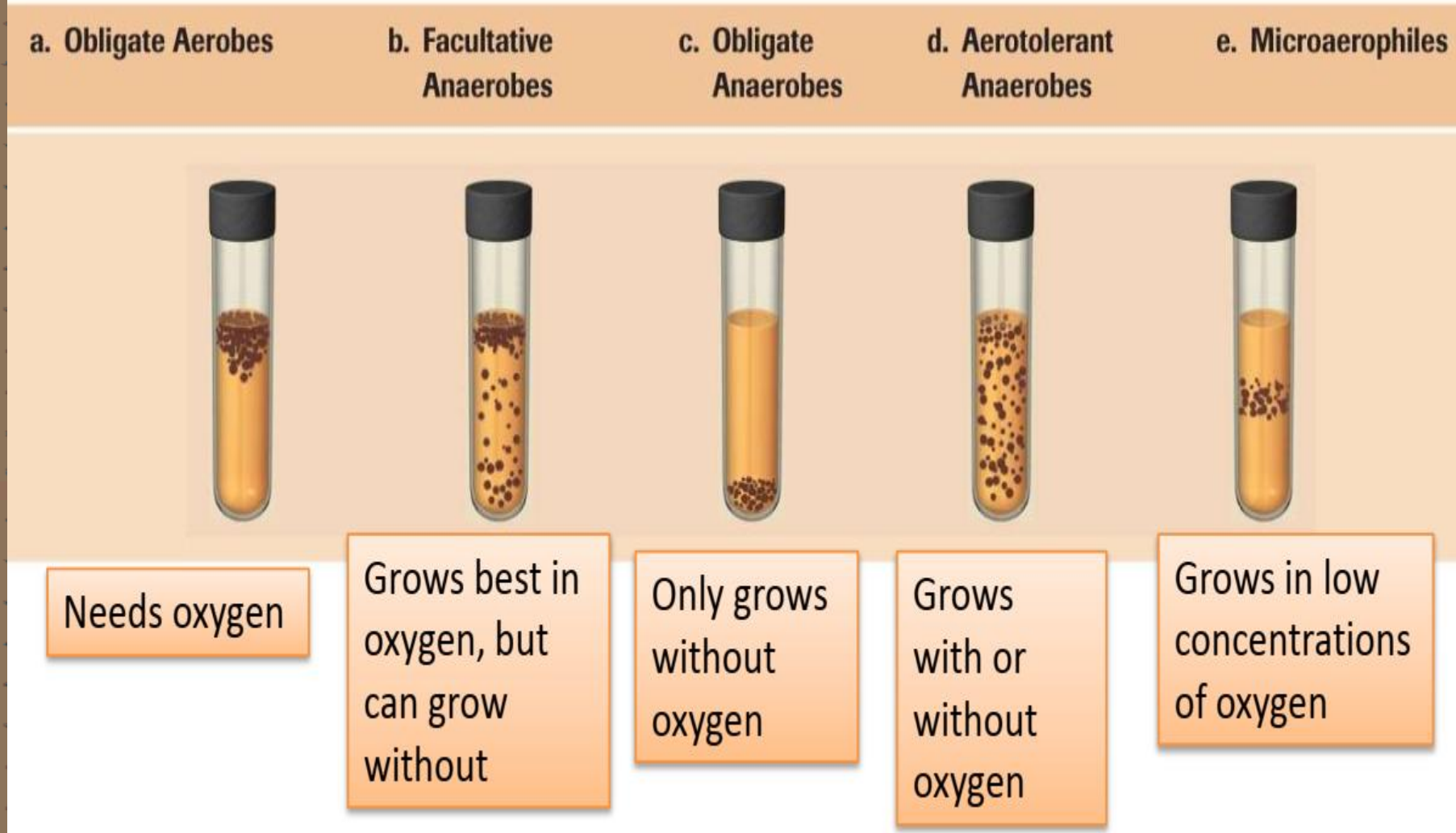
Carbon Dioxide Requirement

All microbes require some carbon dioxide in their metabolism.

- **capneic** – grows best at higher CO_2 tensions than normally present in the atmosphere

(***Brucella abortus***)

The Effect of Oxygen (O2) on Growth



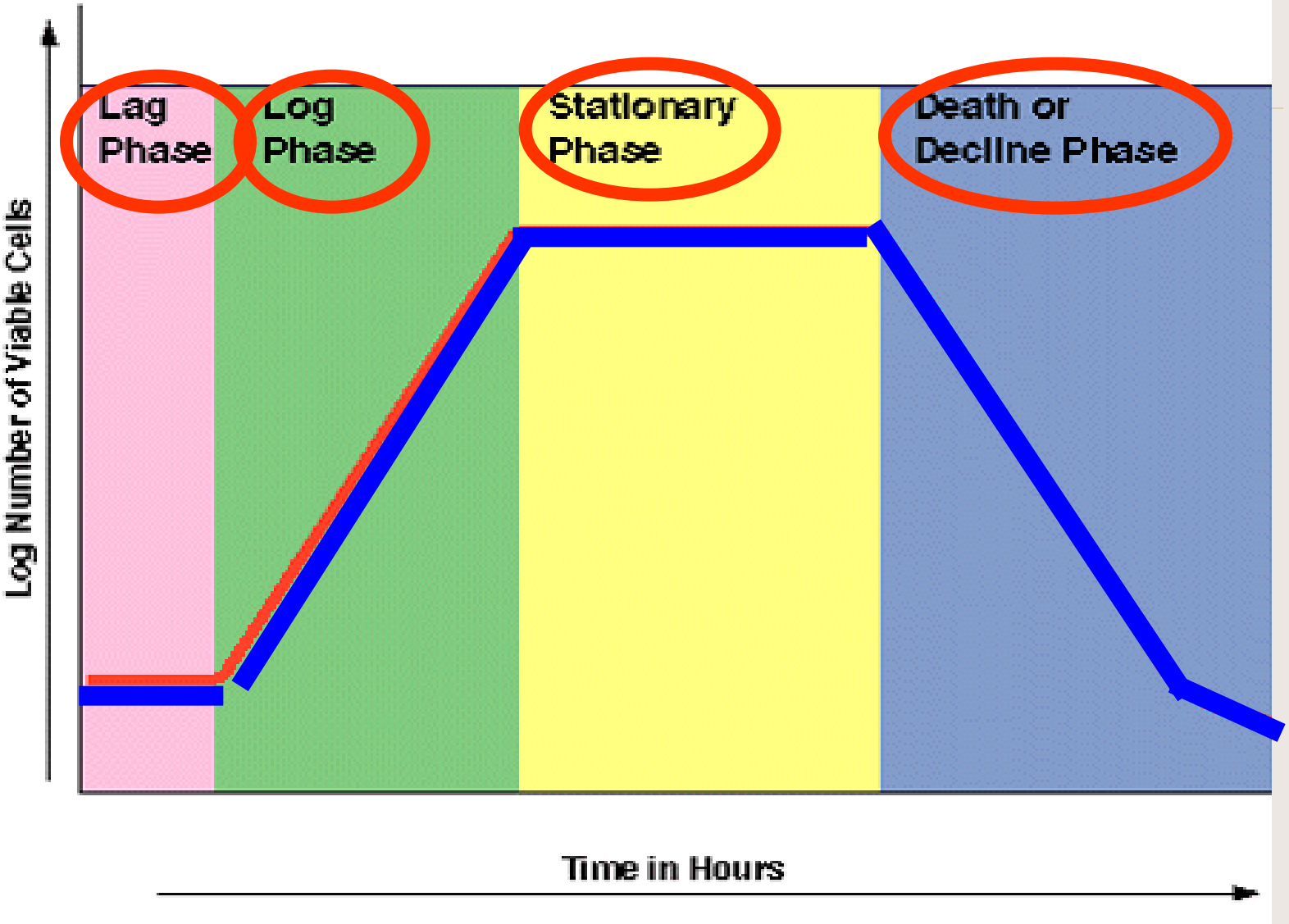
Growth Cycle

Bacterial growth is regulated by nutritional environment. When suitable environment is there that time bacterium is incubated, its growth leads to increase in number of cells which allow definite course.

The growth curve has got four phases:

- Lag phase
- Log phase(logarithmic) or exponential phase
- Stationary phase
- Decline phase

BACTERIAL GROWTH CURVE



Lag phase(1- 4 hour)

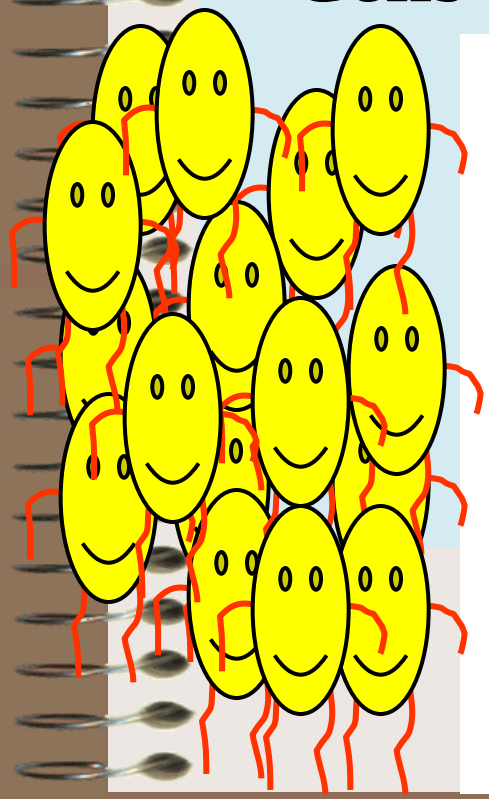
- bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs.
  Length of this phase depend on type of bacterial spesis, culture medium, and environmental factors.

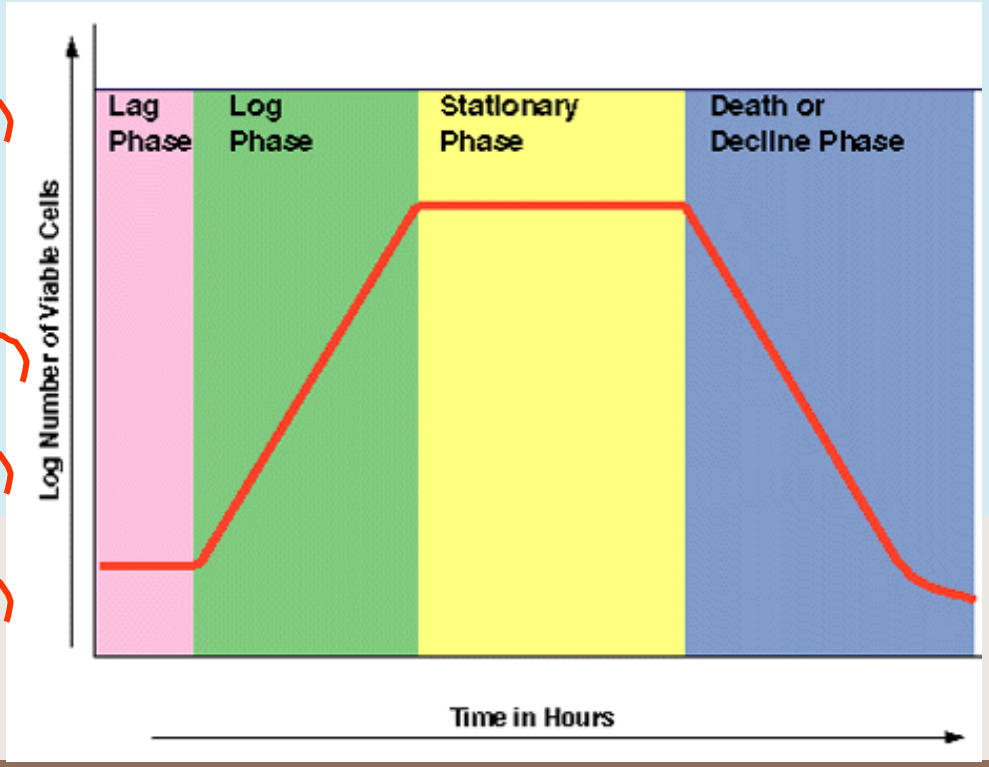
Log phase(logarithmic) or exponential phase (8 hr.)

- it is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line.
- Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.

LOGARITHMIC PHASE

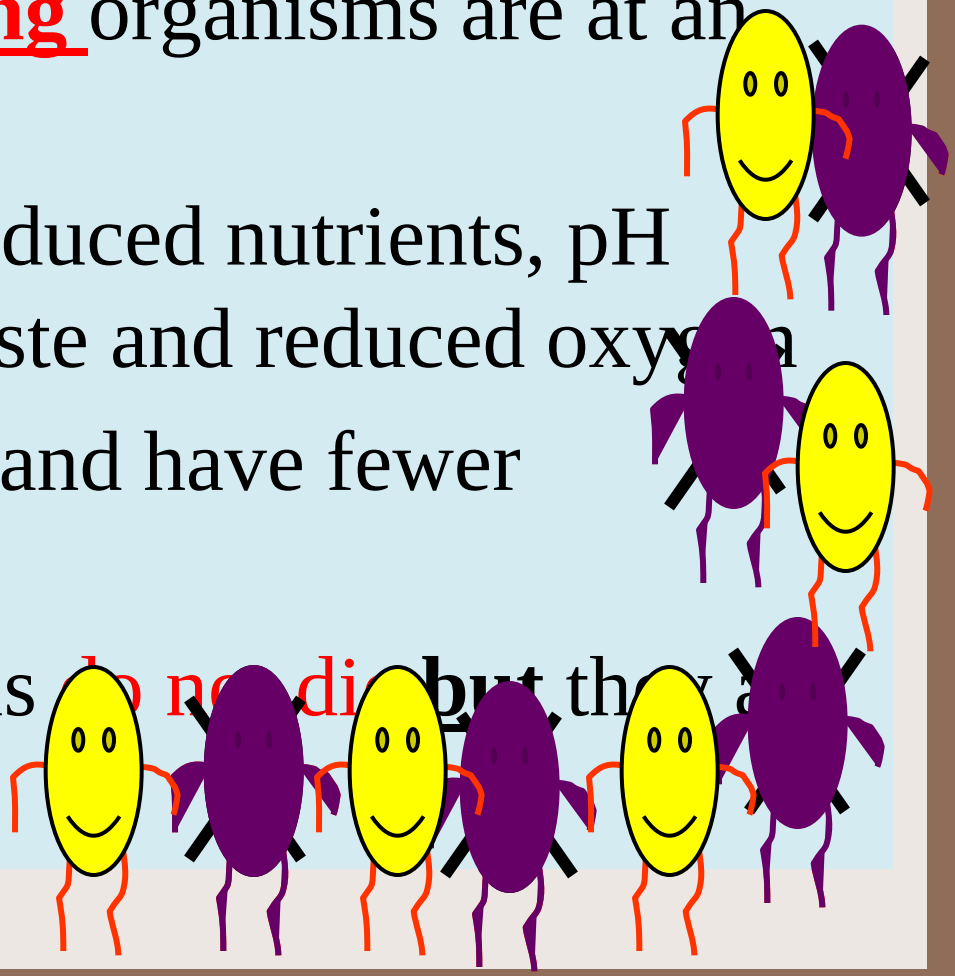
- Division is at a constant rate (**generation time)**
- Cells are most **susceptible** to inhibitors





STATIONARY PHASE

- **Dying** and **dividing** organisms are at an equilibrium
- **Death** is due to reduced nutrients, pH changes, toxic waste and reduced oxygen
- Cells are **smaller** and have fewer ribosomes
- In some cases cells do not die **but** they are not multiplying



DEATH PHASE

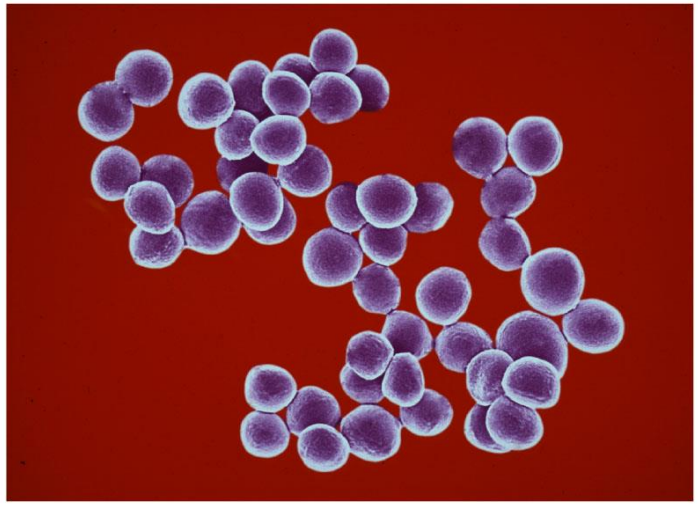
Phase of Decline – population decreases due to the death of cells – autolytic enzymes.

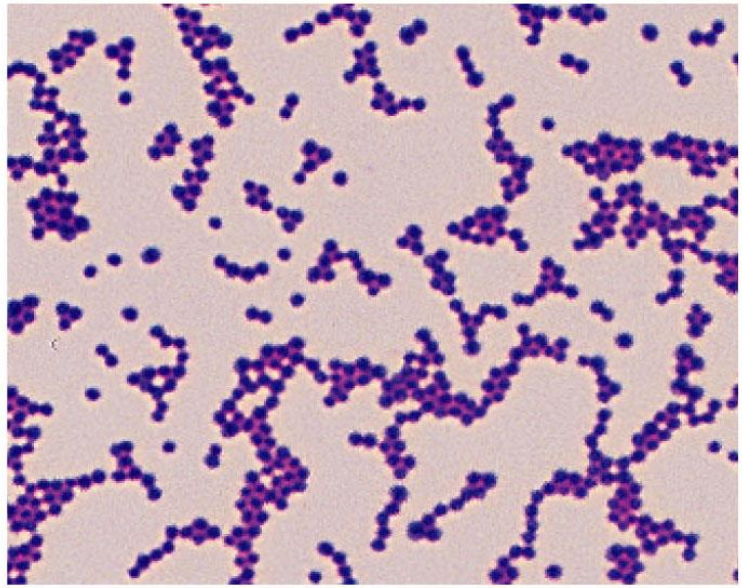
Some cells remain survive at the expense of nutrients released from cell death.

Thank you

Staphylococci

Pathogenic Gram-Positive Cocci (Staphylococci)





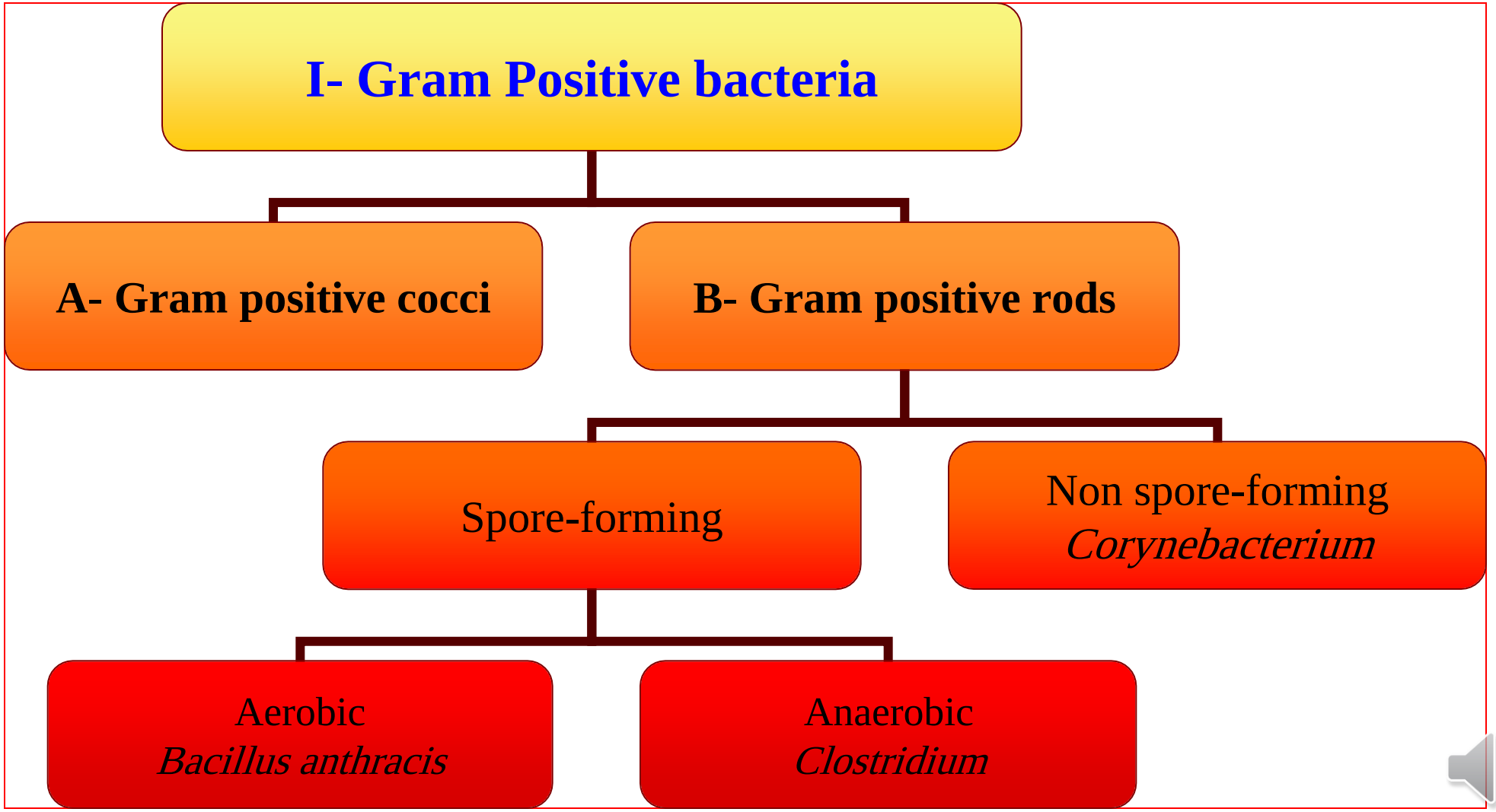
(e)

Presented by:
Shaymaa H. Al-Kubaisy
B.Sc. M. & Ph. D. Med. Microbiology

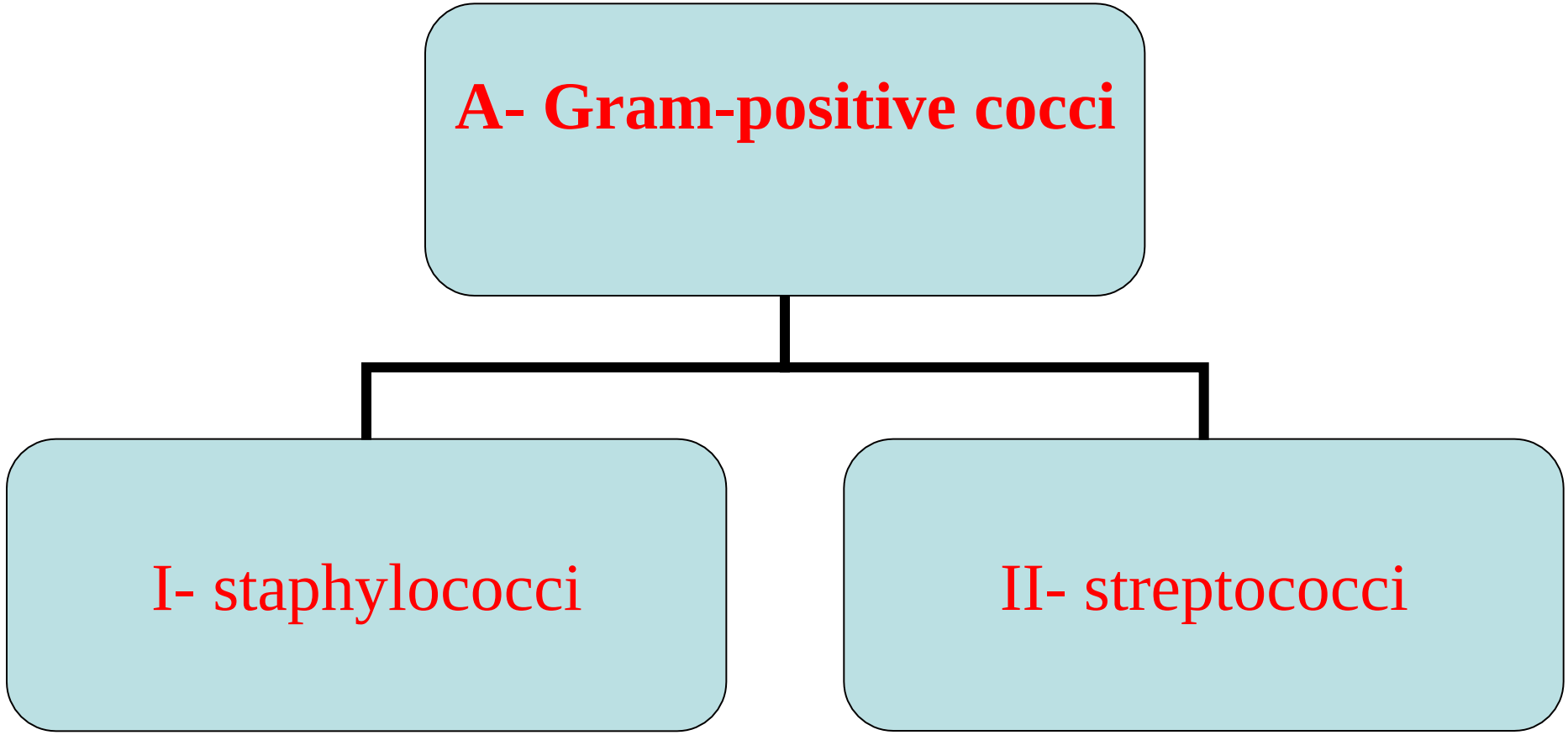
content

- Gram-Positive Pathogens
- * Virulence Factors
- * Pathology
- *Laboratory Diagnosis
- *Resistance of Staphylococci to antimicrobial drugs
- *Treatment

Gram-Positive Bacteria



Gram-Positive Cocci

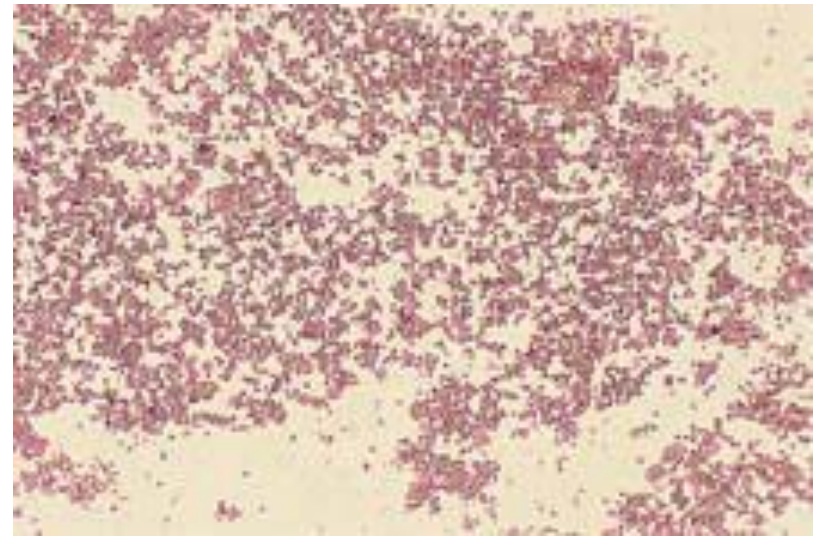


Gram-Positive Pathogens

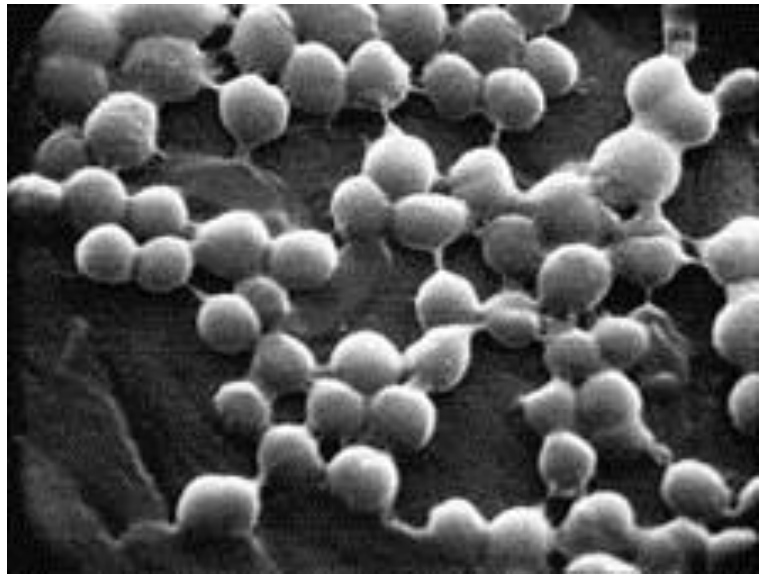
- Stain purple when gram-stained
- Can be categorized into 2 major groups
  - Genera of cocci-shaped organisms- *Staphylococcus*, *Streptococcus*, and *Enterococcus*
  - Genera of bacilli-shaped organisms- *Bacillus*, *Clostridium*, *Listeria*, *Corynebacterium*, *Mycobacterium*, *Propionibacterium*, *Nocardia*, and *Actinomyces*

Staphylococcus: General Characteristics

- Gram-positive spherical cells (0.5-1.5 μm) in singles, pairs, and clusters
- Appear as “bunches of grapes”



Gram-stained smear of staphylococci from colony



Scanning electron micrograph of staphylococci

Staphylococcus: General Characteristics

- Nonmotile
- Non–spore-forming
- Nonencapsulated
- Catalase-producing

Species of Satphylococci

- **Three species of staphyloccoci have medical importance:**
  - ***S. aureus*: Pathogenic & commensally found in nose (nares)**
  - ***S. epidermidis*: non pathogenic & common commensals in nares & skin**
  - ***S. saprophyticus*: Cause UTI in female & occasionally commensally found skin**

Coagulase-Negative Staphylococci

- *S. epidermidis*
- *S. saprophyticus*
- *S. haemolyticus*
- *S. lugdunensis*
- *S. kloosii*
- *S. saccharolyticus*
- *S. simulans*

Staphylococci

General characters:

- Gram Positive Cocci
- Grape-like
- Non Motile
- Non Spore Forming
- Non Capsulated
- Non Fastidious
- Facultative Anaerobes
- Fermentative
- Catalase positive

Characters of *S. aureus*

- ***Production of coagulase***
- ***Production of phosphatase***
- ***Production of DNase***
- **Ferment Mannitol**
- **Gelatin liquefied**
- **B-hemolysis on blood agar**
- **Acidification & clotting of litmus milk**

Characters of *S. aureus*

- ***Staph. aureus*** are usually identified by their tolerance of 7.5% sodium chloride in culture media, the production of a yellow pigment that may vary toward an orange colour, fermentation of mannitol, haemolysis of blood, and coagulation of plasma.

Virulence Factors: Extracellular Enzymes

- **Hemolysins: hemolyze RBCs**
  - **Alpha,Beta , Gamma, Delta: less lethal**
- **Leukocidin :**are capable of destroying leukocytes, and they confer resistance to phagocytosis.
- **Enterotoxins**: There are **four** enterotoxins produced by ***Staph. aureus*****,** and these differ antigenically from one another. The food poisoning toxins cause acute gastroenteritis 2-5 hours **after ingestion**, with the sudden onest of diarrhoea and vomiting.

Virulence Factors: Extracellular Enzymes

- **Coagulase**: **Most of the pigmented staphylococci that have the capacity to cause disease produce factors that clot blood plasma and these are referred to as coagulase. Staphylococcal coagulase is generally regarded as an excellent indicator of potential pathogenicity.**

Toxic Shock Syndrome Toxin

- Most *S aureus* strains isolated from patients with toxic shock syndrome produce a toxin called toxic shock syndrome toxin-1 (TSST-1), which is the same as enterotoxin F. TSST-1 is the prototypical superantigen. TSST-1 binds to major histocompatibility class (MHC) class II molecules, yielding T-cell stimulation, which promotes the protean manifestations of the toxic shock syndrome.

.

TSST-1: Toxic shock syndrome toxin-1

- Multisystem disease involvement, including adesquamative skin rash.
- High fever
- Hypotension
- Shock

Virulence Factors: Extracellular Enzymes

- Hyaluronidase: **(spreading factor) which depolymerizes hyaluronic acid, the mucopolysaccharide of the intercellular cement substance of tissues.**
- Staphylokinase: fibrinolysin (to dissolve fibrin clot).
- Lipase: allows colonization
- Penicillinase: confers resistance

PATHOLOGY

- **T**he pathologic picture of a staphylococcal infection is a **localized abscess**. Inflammatory cells, including leukocytes, gather about the lesion.

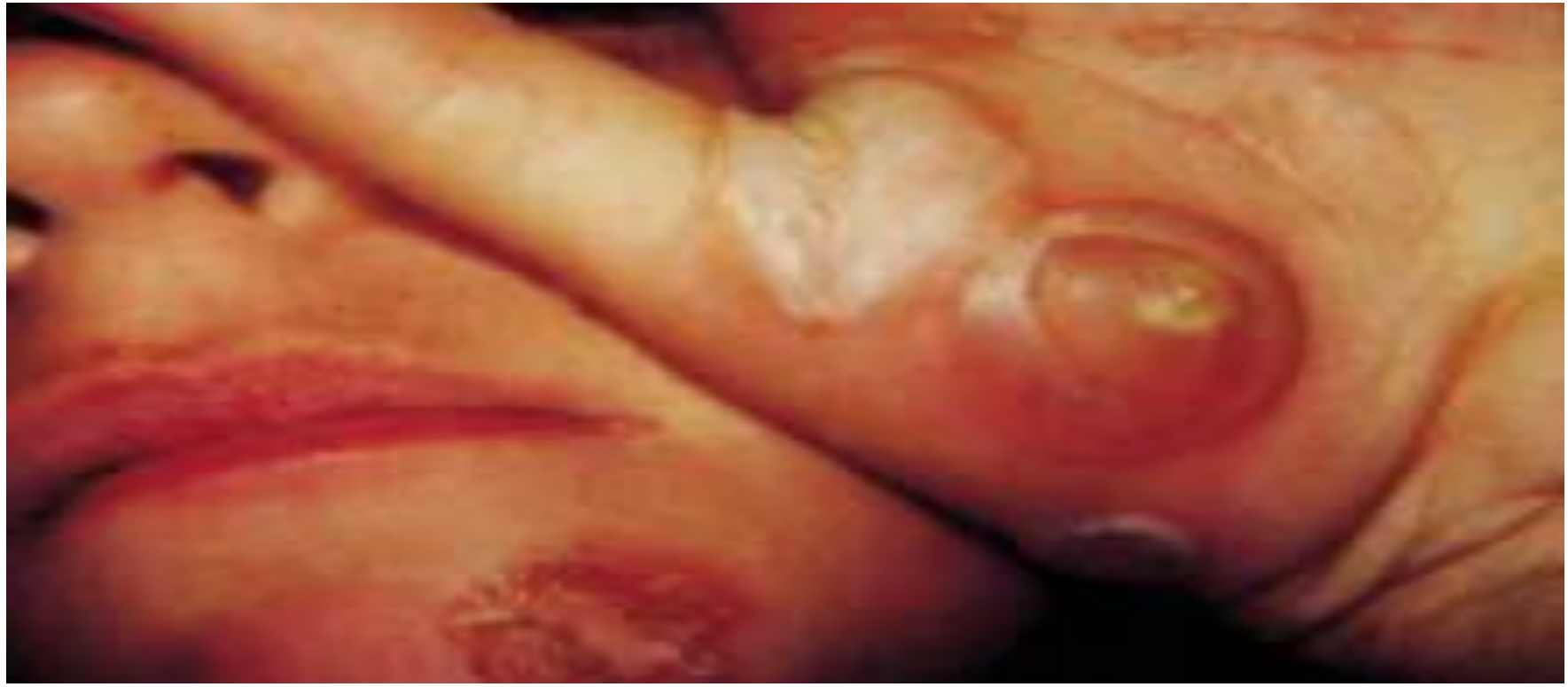
- the term **pyogenic infection**.
- spread from one site to others by way of the lymphatic or blood vessels to set up new abscesses.
- major cause of hospital acquired **(nosocomial**) infection
- **food poisoning** by releasing enterotoxins into food and toxic shock syndrome by release of superantigens into the blood stream.

- **Staphylococcal pneumonia** is a frequent complication of influenza.
- More serious infections of the skin may occur, such as furuncles or impetigo. Localized infection of the bone is called **osteomyelitis**

Disease caused by *S. aureus*

- Localized suppurartive (Pyogenic) inflammation:
  - Folliculitis → Infection of hair follicles
  - Furuncle → Infection of an obstructed hair follicle
  - Carbuncle → Larger abscess
  - Deep Lesions (Osteomyelitis, Endocarditis & Meningitis)
- Toxigenic infection
  - Scalded Skin Syndrome (SSS)
  - Toxic Shock Syndrome
- Food poisoning
  - Nausea, Vomiting, Diarrhea without Fever within 8 h after ingestion of toxins in the contaminated food

Staphylococcus aureus: Clinical Infections



Bullous impetigo

Staphylococcus aureus: Clinical Infections

- Other infections
  - Respiratory (less often)
  - Bacteremia
  - Osteomyelitis

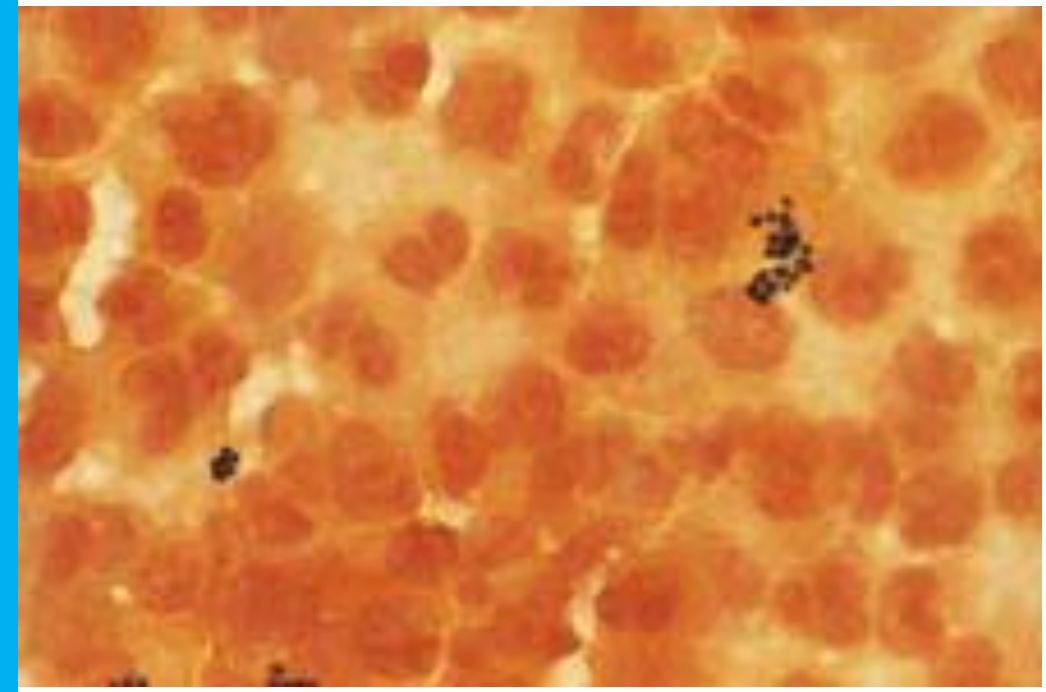
Laboratory Diagnosis: Specimen Collection and Handling

- Samples must be taken from the actual site of infection
- Prevent delay in transport of collected material from infected sites
- Transport in appropriate collection device that would prevent drying and minimize growth of contaminating organisms

Laboratory Diagnosis: Direct Smear Examination

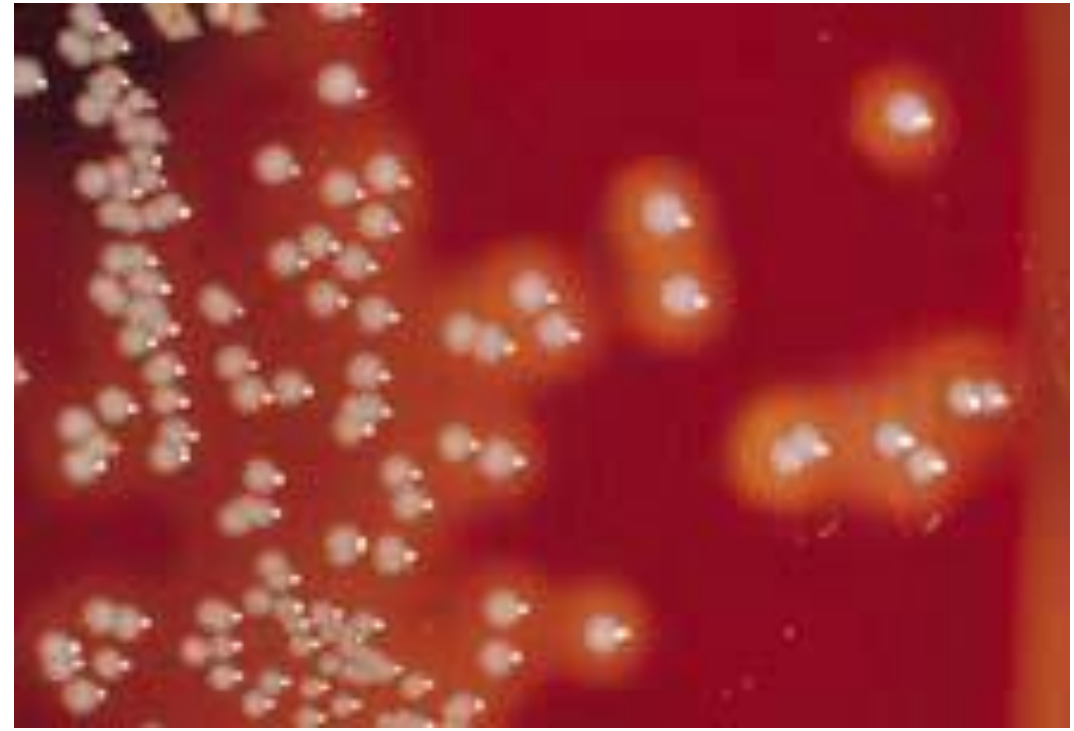
Microscopic Examination

- Gram-positive cocci
- pairs and clusters
- Numerous polymorphonuclear cells (PMNs)



Laboratory Diagnosis:Cultural Characteristics

- Colony morphology
  - Smooth white to yellow, creamy
  - *S. aureus* may produce hemolysis on blood agar



S. aureus

Laboratory Diagnosis: Cultural Characteristics

- **Coagulase-negative staphylococci**
  - Smooth, creamy, white
  - Small-to medium- sized, usually non-hemolytic
- ***S. saprophyticus***
  - Smooth, creamy, may produce a yellow pigment

Identification Tests: Catalase

- Principle: tests for enzyme catalase

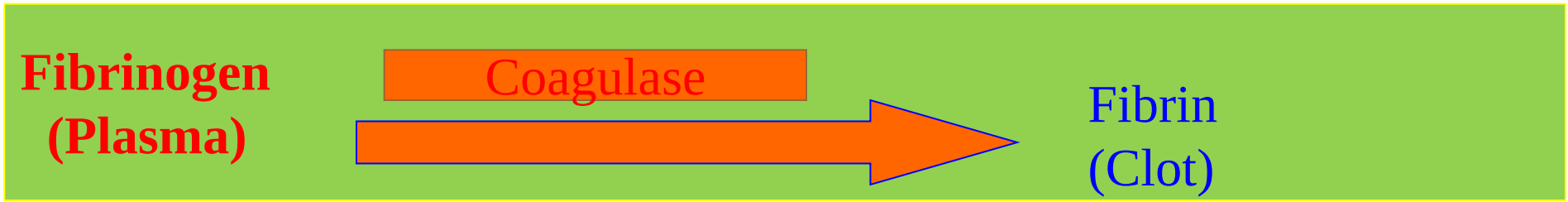
$$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2$$

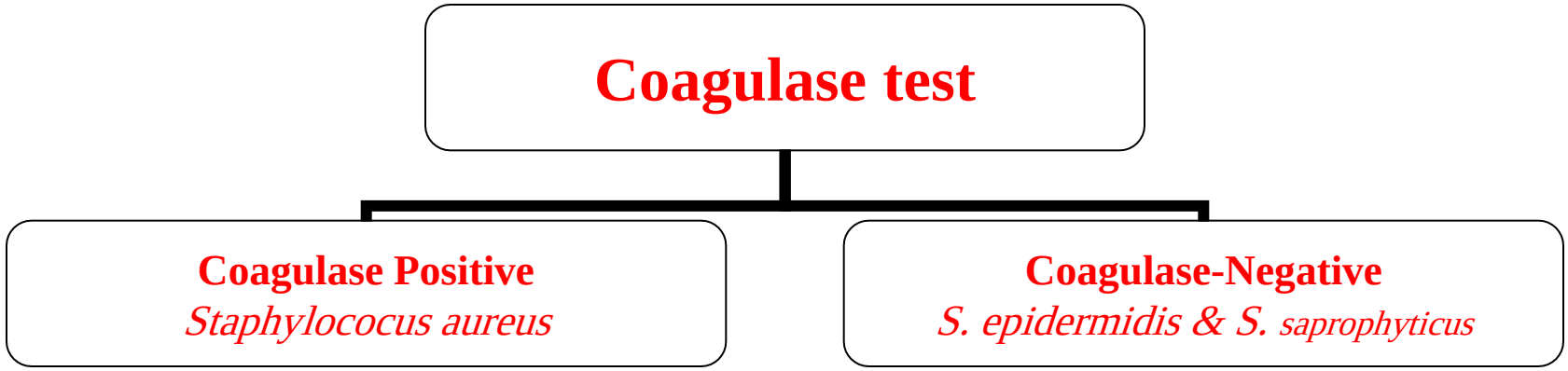
- Drop H_2O_2 onto smear
- Bubbling = **POS** (Most bacteria, O_2 generated)
- No bubbling = **NEG** (Streptococci and other lactic acid bacteria, no O_2 generated)

Coagulase Test

Principle:

- This test used to differentiate between *S. aureus* & other





Identification Tests: Coagulase Test

Tube test detects the extracellular enzyme “free coagulase”

Resistance of Staphylococci to antimicrobial drugs

- Resistance is caused by several mechanisms:
- 1. β-Lactamase production is common, is under plasmid control, and makes the organisms resistant to many penicillins (penicillin G, mpicillin, ticarcillin, piperacillin and similar drugs). The plasmids are transmitted by transduction and perhaps also by conjugation.
- 2. Resistance to nafcillin (and to methicillin and oxacillin) is encoded and regulated by a sequence of genes found in a region of the chromosome called the staphylococcal cassette chromosome *mec* (*SCCmec*).
-

- 3-These are often known as **vancomycin-intermediate *S aureus* (VISA)**. They generally have been isolated from patients with complex infections who have received prolonged vancomycin therapy. Often there has been vancomycin treatment failure. The mechanism of resistance is associated with increased cell wall synthesis and alterations in the cell wall .

- 4-several isolates of vancomycin-resistant *S aureus* (VRSA) strains were isolated from patients in the United States. The isolates contained the vancomycin resistance gene vanA from enterococci and the nafcillin resistance gene mecA. Both of the initial VRSA strains were susceptible to other antibiotics. Vancomycin resistance in *S aureus* is of major concern worldwide.

- **5-** Plasmid-mediated resistance to tetracyclines, erythromycins, aminoglycosides, and other drugs is frequent in staphylococci.
- 6-"Tolerance" implies that staphylococci are inhibited by a drug but not killed by it—that is, there is great difference between minimal inhibitory and minimal lethal concentrations of an antimicrobial drug.

- A **plasmid** associated with vancomycin resistance has been detected in *Enterococcus faecalis* which can be transferred to *Staph. aureus* ***in*** **the laboratory**, and it is speculated that this transfer **may occur naturally** (e.g. gastrointestinal tract).
- In addition, *Staph. aureus* exhibits resistance to **antiseptics and disinfectants**, such as quaternary ammonium compounds, which may aid its survival in the hospital environment.

Treatment

- Vancomycin is often reserved for use with nafcillin-resistant staphylococci. In recent years, an increase in MICs to vancomycin among many MRSA strains recovered from hospitalized patients has led physicians to seek alternative therapies.
- Alternative agents for the treatment of MRSA bacteremia and endocarditis include newer antimicrobials such as daptomycin, linezolid, and quinupristin–dalfopristin

Listeria Monocytogenes

Represented by:-
Lecturer Dr. Shaymaa H. Al-Kubaisy
B.Sc. M.Sc. Ph.D. Med. Microbiol.

Listeria monocytogenes

short , motile, non-spore - forming, Grm + rods, β - hemolytic, looks like Group B streptococcus but group B is catalase neg and non-motile.

found in wild and domestic animals excreted into soils.

healthy human carriers 5-10 %.

source of infection is food and animal contact.


Listeria monocytogenes

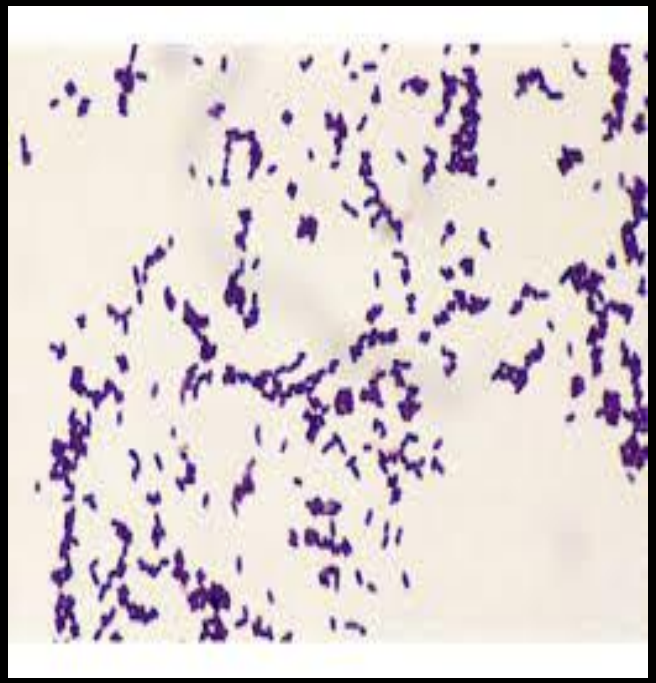
Listeria Infections

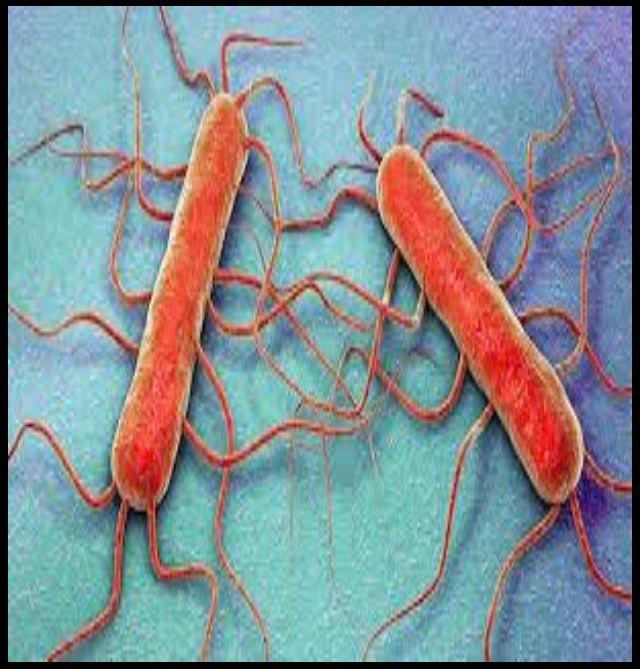
- Neonatal sepsis or meningitis
- Spontaneous abortion or still birth
- Sepsis or meningitis in immunocompromised patients
- Puerperal sepsis

Persons at risk: Pregnant, post partum, new born, organ transplants

WHAT IS *LISTERIOSIS*?

- ***Listeriosis*** **is a serious infection caused by eating foods contaminated with the bacterium *Listeria Monocytogenes.***
- **This disease affects primarily pregnant women, newborn, and adults with weakened immune systems.**
- **This bacterium is too small to be seen without a microscope.**

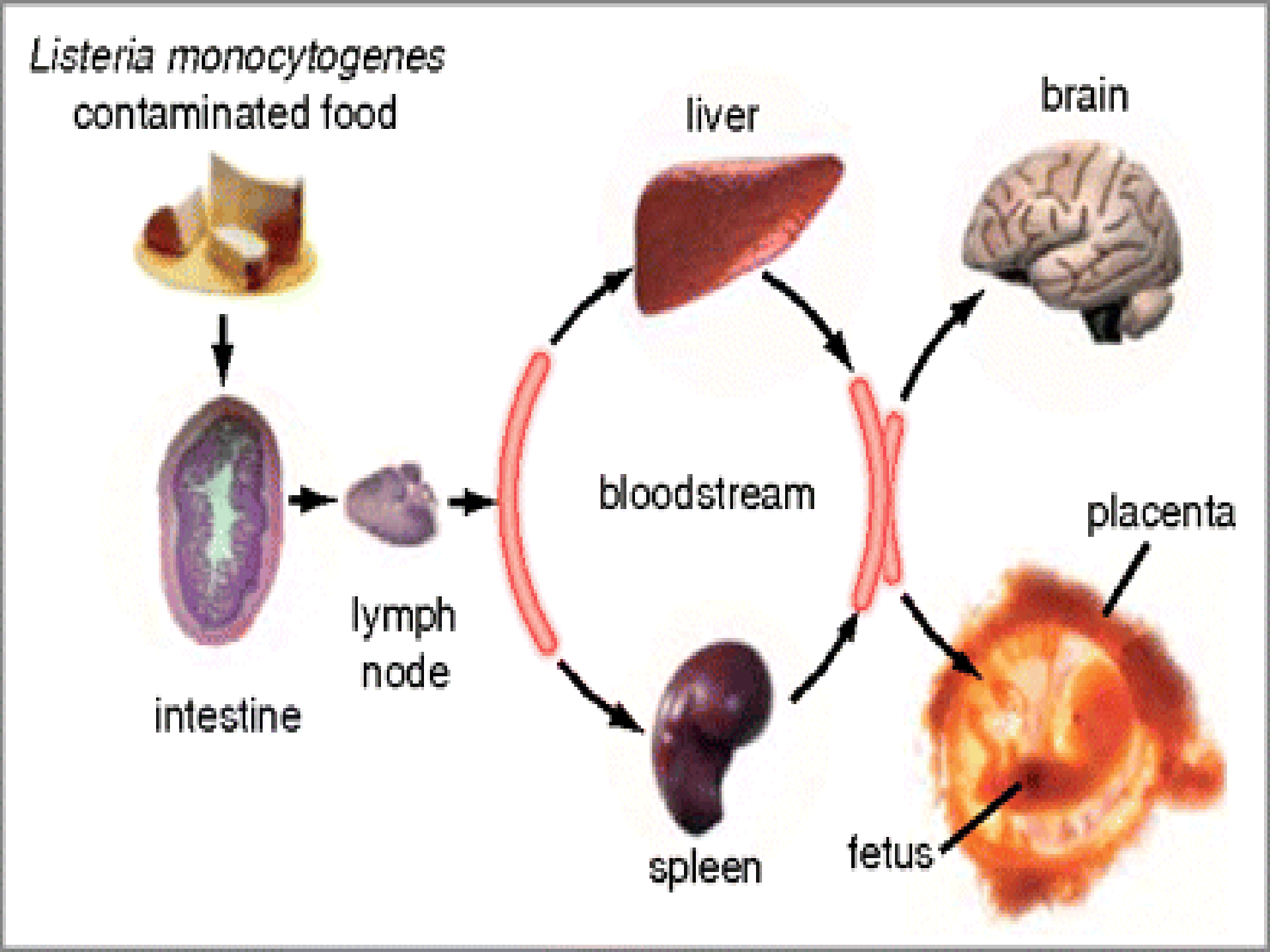




Listeria monocytogenes **is a rod-shaped aerobic and gram positive pathogenic bacterium that invades the cytoplasm of living cells. It develops a distinctive rocket tail structure to help push through the cytoplasm. Eventually, these "rockets" push bacteria into neighboring cells, propagating the infection**

Listeria Pathogenesis

- Ingested raw contaminated food
- Penetrates cell of intestine
- common event
- most have T cells
- Facultative intracellular pathogen
- Immunocompromised host
- Transmitted congenitally


Listeria monocytogenes
contaminated food
liver
brain
bloodstream
placenta
lymph
node
intestine
spleen
fetus

SYMPTOMS

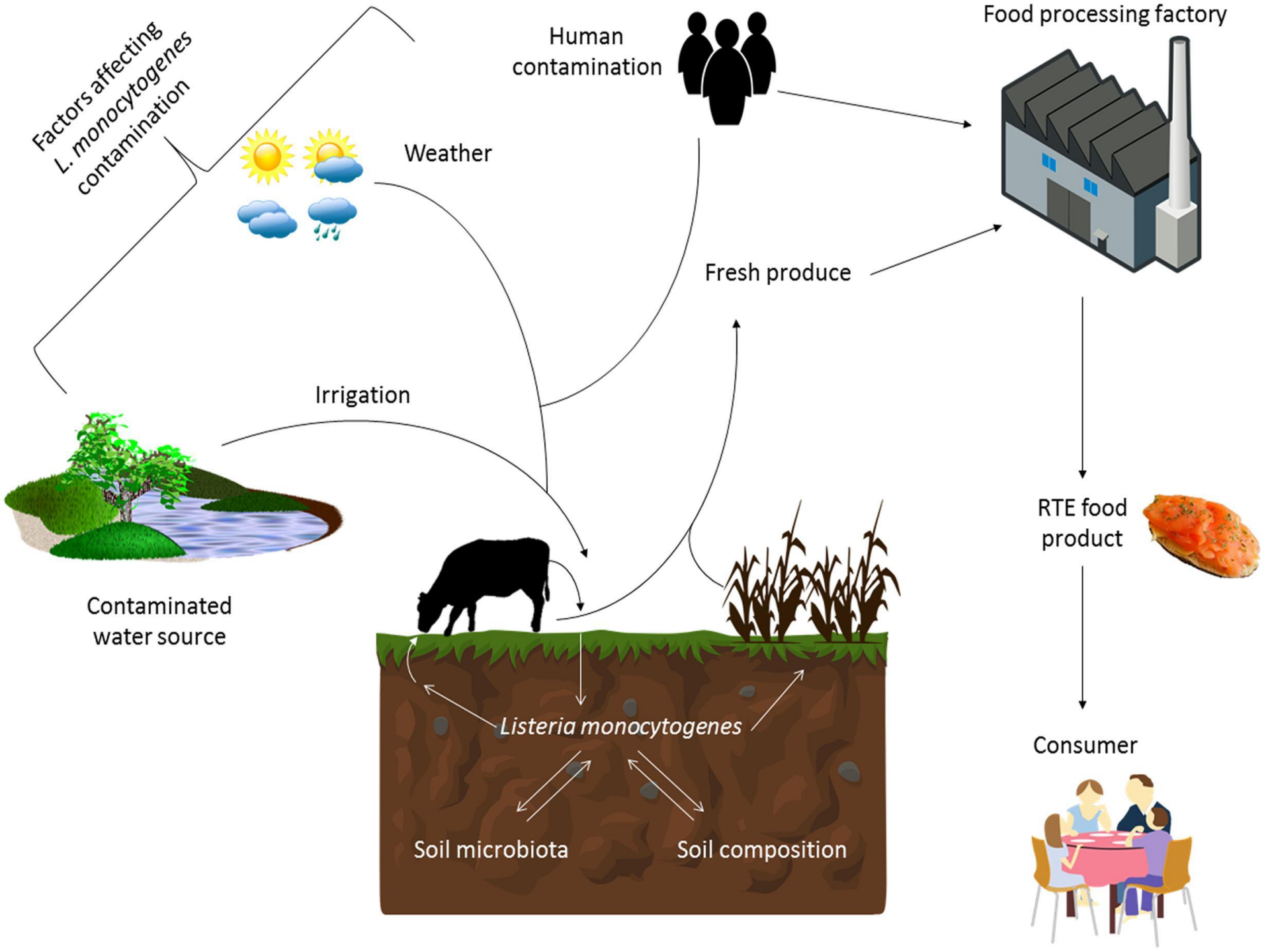
- Fever
- Muscle ache
- GI Sx: Nausea, diarrhea
- Pregnant women: mild flu-like Sx, miscarriage, still birth, premature delivery, or infected newborn.
- Lethargy
- irritability

- If infection spreads to the nervous system: headache, stiff neck, confusion, loss of balance, or convulsions.
- Listeria can cause Pneumonia, Meningitis, and Sepsis.

CONTAMINATION

- ***Listeria Monocytogenes*** **is found in soil and water.**
- **Vegetables can become contaminated from the soil or from manure used as fertilizer.**
- **Animals can carry the bacterium without appearing ill and can contaminate foods of animal origin such as meats and dairy products.**

- **The bacterium has been found in uncooked meats and vegetables, soft cheeses and unpasteurized milk or foods.**
- **If acquired at birth, the incubation period is 7 to 28 days.**
- **The average incubation period is 31 days; with a range from 11 to 47 days.**


Factors affecting
L. monocytogenes
contamination
Human
contamination
Food processing factory
Weather
Fresh produce
Irrigation
Contaminated
water source
RTE food
product
Listeria monocytogenes
Soil microbiota
Soil composition
Consumer

Food poisoning

MILK and dairy products. This species grows at refrigerator temp. 5-12^{o} C

Isolated by cold enrichment.

DIAGNOSIS

- There is no routine screening test for susceptibility.
- If you have Sx of fever, or stiff neck, consult your doctor.
- A blood or spinal fluid test (to cultivate the bacteria) will show if you have *Listeriosis*.
- During pregnancy, a blood test is the most reliable way to find out if your Sx are due to *Listeriosis*.

Test for Listeria in Milk

Add fluorescent-labelled antibodies specific

for *Listeria monocytogenes* to milk

Pass through a flow cytometer. fluid is passed through a small opening

Listeria detected by a laser beam

NO CULTURE NEEDED!!!

Listeriosis Prevention

Persons at risk

- avoid soft cheeses and unpasteurized milk and processed meat that cannot be cooked.
- thoroughly cook all meats especially hot dogs
  - left over meat should be cooked to steaming.

TREATMENT

- ***Listeriosis*** **is a serious disease requiring hospitilization.**
- **A combination of antibiotics is given intravenously through a small straw-like catheter.**
- **When infection occurs during pregnancy, antibiotics must be given promptly to the mother to prevent infection of the fetus or newborn.**
- **Babies with *Listeriosis* receive the same antibiotics as adults.**
- **The duration of antibiotic treatment is at least 2 weeks.**
- **Even with prompt treatment, some infections result in death.**

Listeria Monocytogenes

- Gram Positive Bacilli, Facultative intracellular rod, Catalase positive, Beta hemolytic.
- Shows Tumbling motility at 20–25°C but at 37°C its nonmotile.

Transmission:

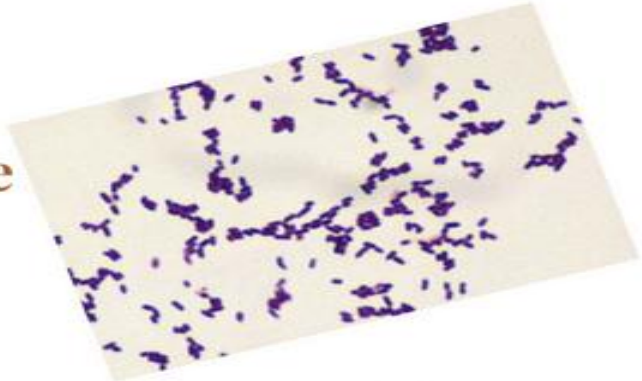
- By ingestion of unpasteurized dairy products, Transplacental transmission from mother to fetus.

Pathogenesis:

- Listeriolysin O is the virulence factor that allows microorganism to escape the phagolytic vacuole inside the host cell.

Culture:

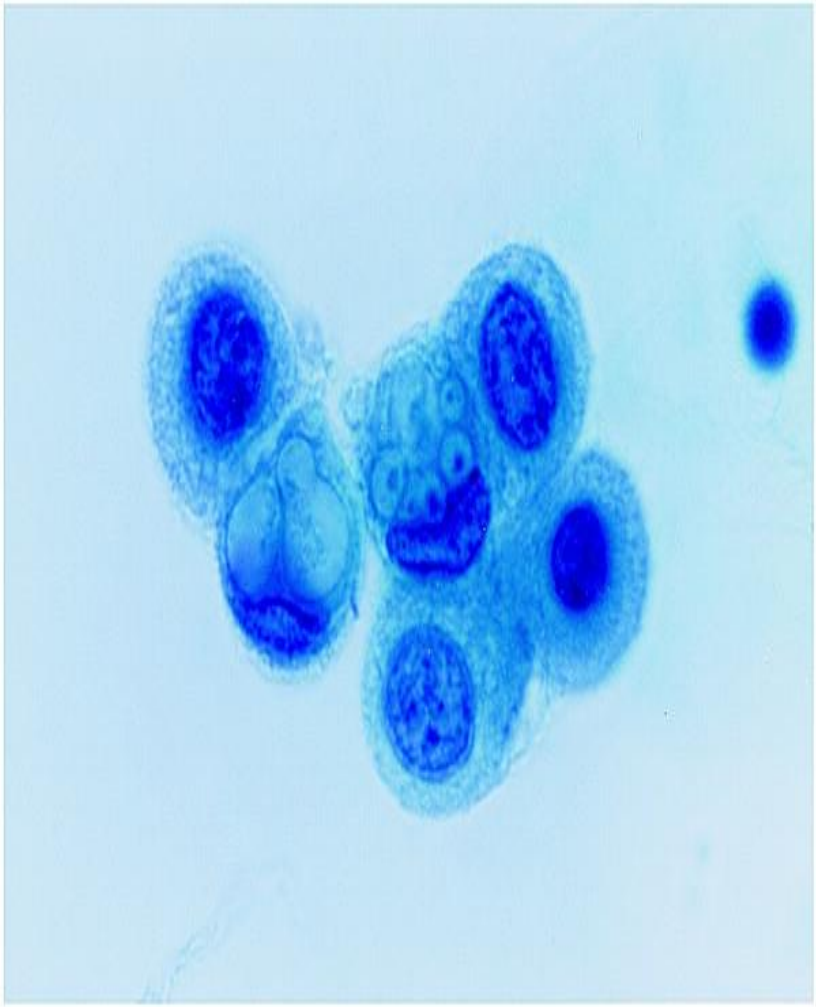
- Grows on ordinary media, Chocolate agar, PALCAM agar.



Diseases Caused:

- Granulomatosis infantisepticum, Neonatal Meningitis, Meningitis in immunocompromised, Mild gastroenteritis.

The End



Chlamydia

Presented by:
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Chlamydiae that infect humans are divided into three species,

Chlamydia trachomatis,

Chlamydia (Chlamydophila)pneumoniae, and

Chlamydia (Chlamydophila) psittaci **,**

on the basis of antigenic composition, intracellular inclusions, sulfonamide susceptibility, and disease production.

Classification:

Three that infect humans

C. trachomatis: This species produces compact intracytoplasmic inclusions that contain glycogen; it is usually inhibited by sulfonamides. It includes agents of human disorders such as trachoma, inclusion conjunctivitis, nongonococcal urethritis, salpingitis, cervicitis, pneumonitis of infants,and lymphogranuloma venereum (LGV).

C. psittaci: This species produces diffuse intracytoplasmic inclusions that lack glycogen; it is usually resistant to sulfonamides. It includes agents of psittacosis in humans, and other animal diseases.

C. pneumoniae: This species produces intracytoplasmic inclusions that lack glycogen; it is usually resistant to sulfonamides. It causes respiratory tract infections in humans.

Th e chlamydiae can be viewed as **gram-negative bacteria**
that lack mechanisms for the production of metabolic energy and
cannot synthesize adenosine triphosphate (**ATP**).
Thus, chlamydiae are **obligate intracellular parasites**

Developmental Cycle

All chlamydiae have unique biphasic developmental cycle. The environmentally stable infectious particle is a small cell called the elementary body (EB).
These are about 0.3 µm in diameter.
The EBs have a high affinity for host epithelial cells and rapidly enter them. **There appear to be multiple adhesins, receptors, and mechanisms of entry.**
Heparan sulfate–like proteoglycans on the surface of *C trachomatis* are likely possibilities for mediating at least the initial interaction between EBs and host cells.

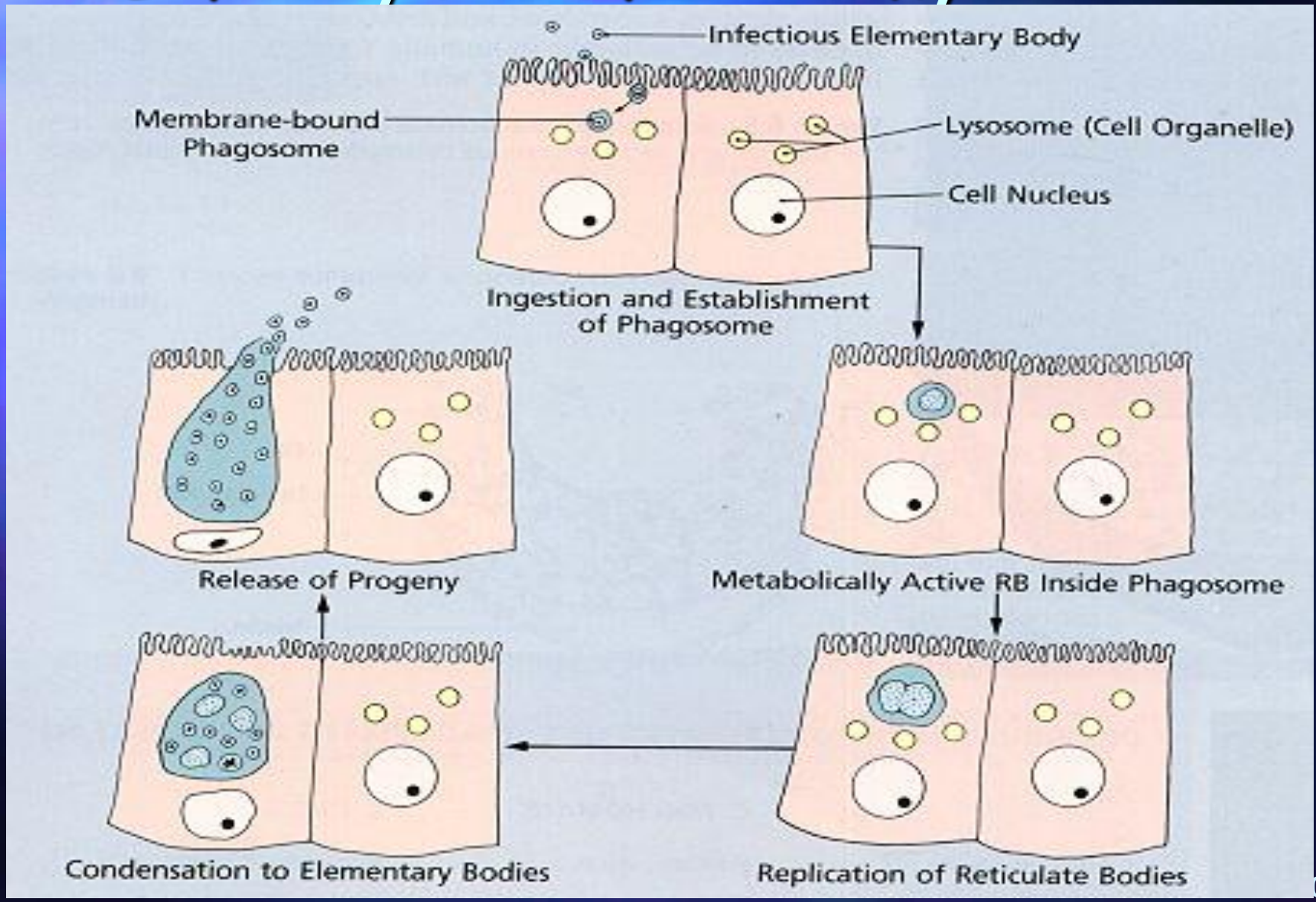
Other potential adhesins include the major outer membrane protein (MOMP) , glycosylated MOMP, and other surface proteins.

The mechanisms thought to mediate entry into the host cell also varied. **EBs are usually seen attached near the base of microvilli,** where they are subsequently engulfed by the host cell

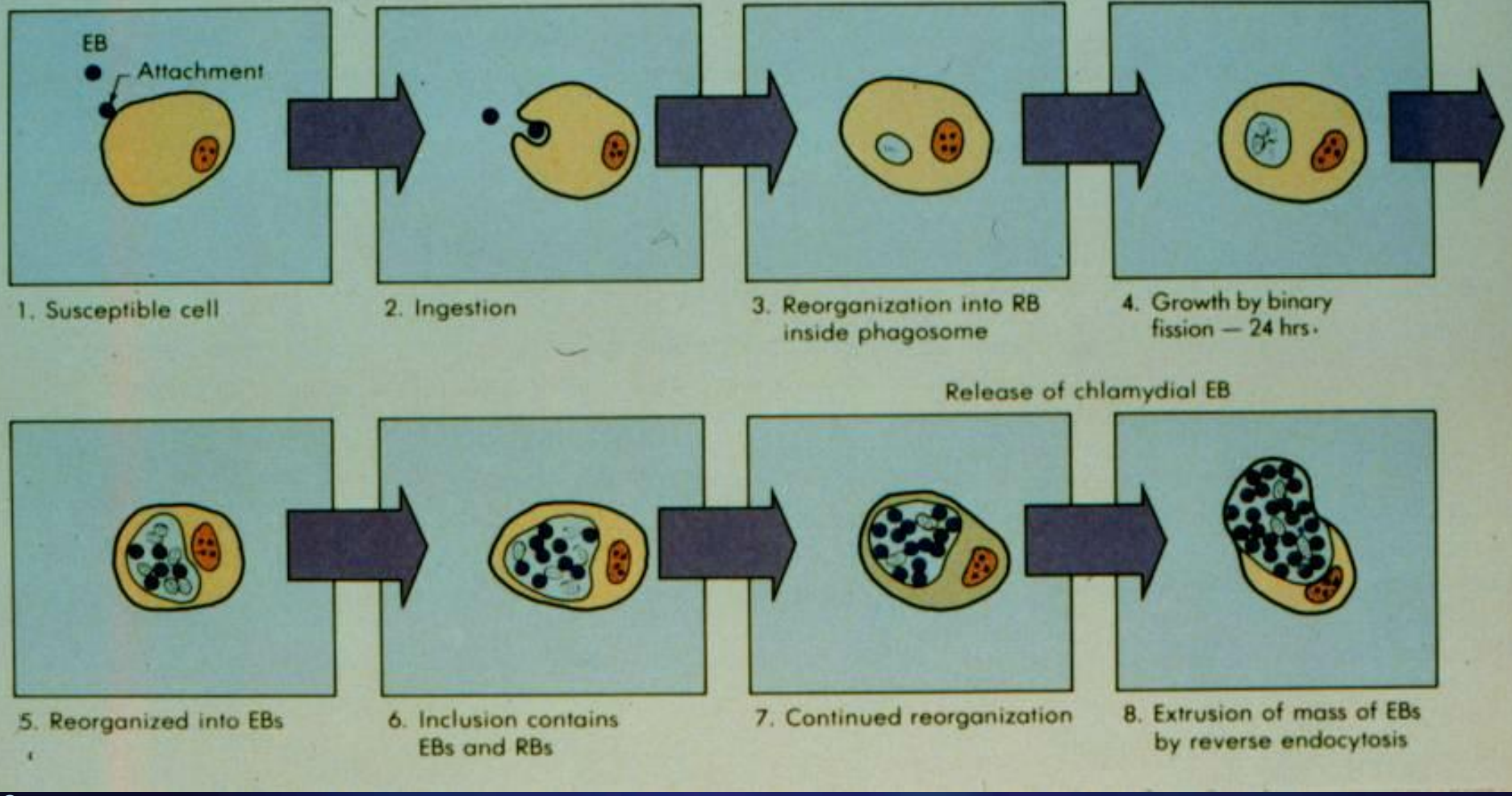
Lysosomal fusion is inhibited, creating a protected membrane-bound environment around the chlamydiae. Shortly after entry Into the host cell

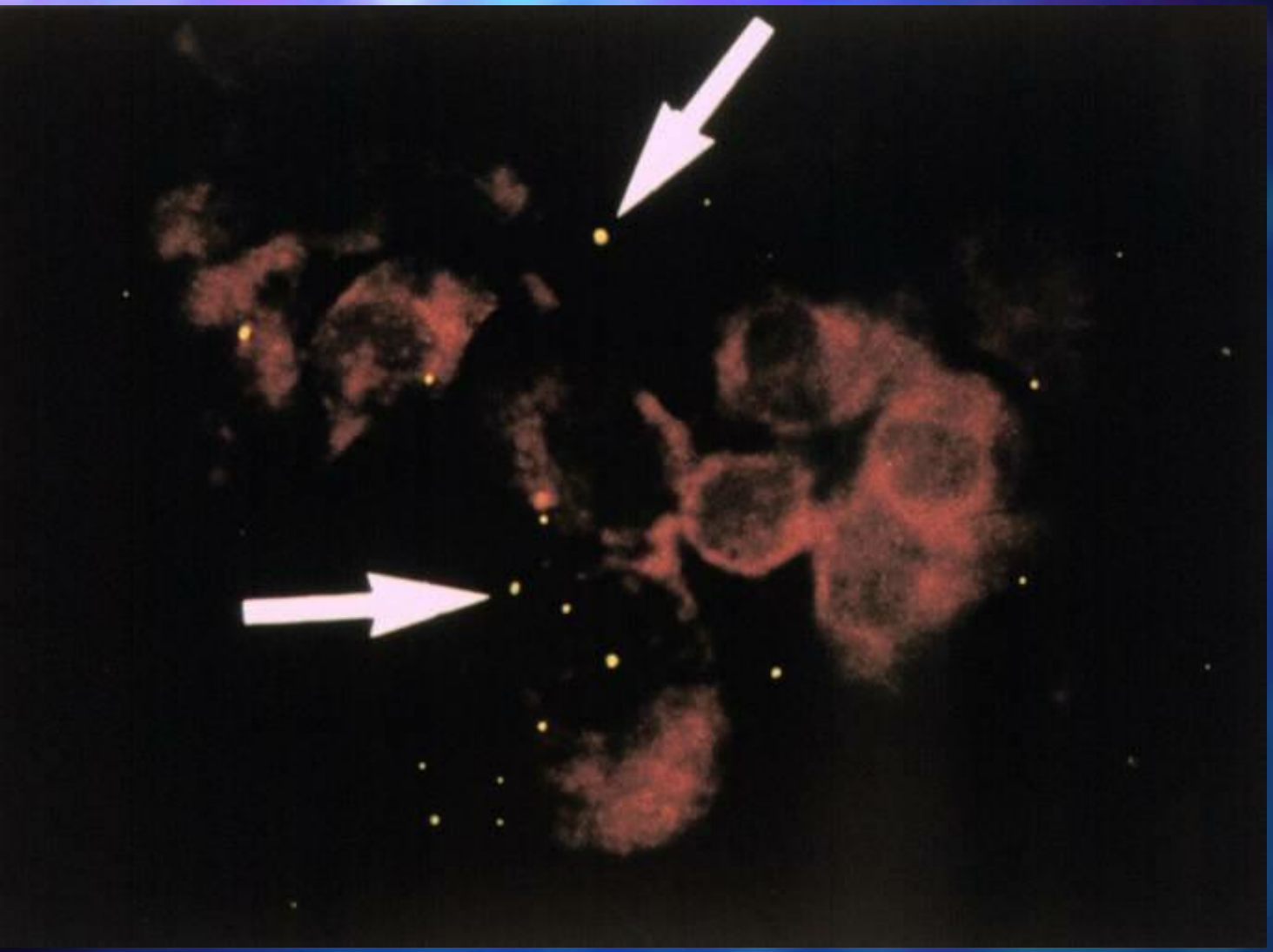
the disulfide bonds of the EB membrane proteins are no longer cross-linked, and the EB is reorganized into a larger structure called a reticulate body (RB) measuring about 0.5–1 μm
the RB grows in size and divides repeatedly by binary fission. Eventually, the entire vacuole becomes filled with EBs derived from the
RBs to form a cytoplasmic inclusion . The newly formed EBs may be liberated from the host cell to infect new cells. The developmental cycle takes 24–48 hours.

Life Cycle of Chlamydia



Developmental Cycle of Chlamydia





C trachomatis elementary bodies

Growth and Metabolism

- Chlamydiae require an intracellular habitat because they are unable to synthesize ATP and depend on the host cell for energy requirements.

- The replication of chlamydiae can be inhibited by many antibacterial drugs. Cell wall inhibitors such as **penicillins and cephalosporins**
- Inhibitors of protein synthesis (**tetracyclines,erythromycins)** are effective

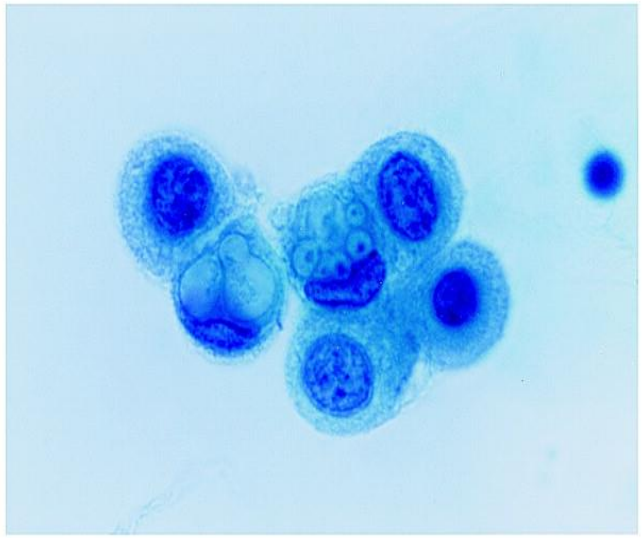
What is chlamydia?

- **Chlamydia is a common STD that can infect both men and women. It can cause serious, permanent damage to a woman's reproductive system.**

This can make it difficult or impossible for her to get pregnant later on.

Chlamydia can also cause a potentially fatal ectopic pregnancy (pregnancy that occurs outside the womb).

STI caused by bacterium
Chlamydia trachomatis
primarily targets cells of mucous membranes including urethra, vagina, cervix and endometrium (mouth and throat)
one of most commonly reported bacterial STDs



Chlamydia

- Obligate intracellular coccoid parasites
- contain DNA and RNA, and ribosomes
- lack ATP, biosynthetic pathways
- cell wall but peptidoglycan absent -
  - use disulfide bonds
- non motile

Chlamydia Characteristics

- Unique growth cycle because they are deficient in independent energy metabolism; therefore they are obligate intracellular parasites
- Replication involves **elementary body (EB)** and **reticulate body (RB)**

Symptoms and signs

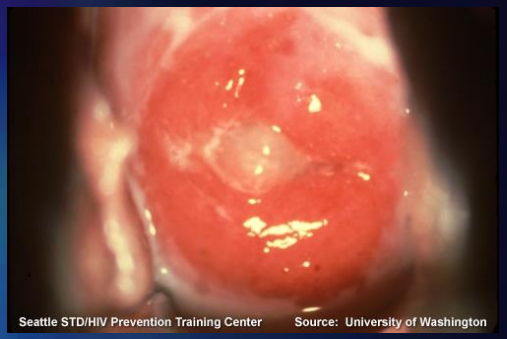
- appear between 1 and 3 weeks after exposure (may not emerge until much later)

-“silent disease”

- 70-75% asymptomatic women

Women

Complications



- **Pelvic Inflammatory Disease (PID)**
  - higher risk of ectopic pregnancy, premature birth, infertility
- **Mother-to-child-transmission (MTCT)**
  - eye or lung infection
- **Cerviciti**

- yellowish vaginal discharge and pain during sex
- deep pelvic pain and backache

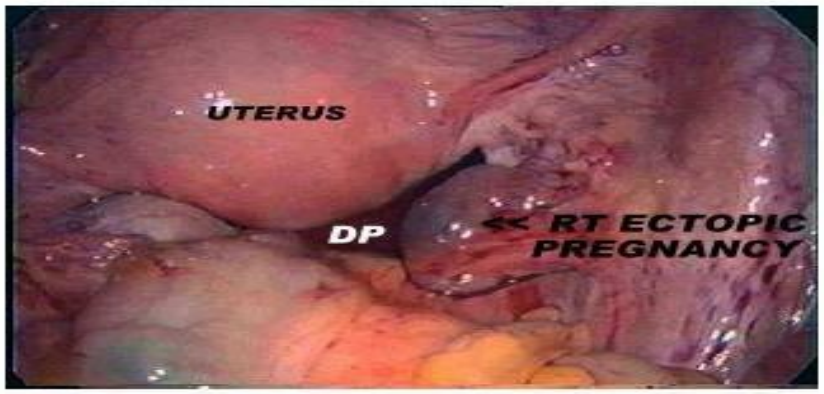


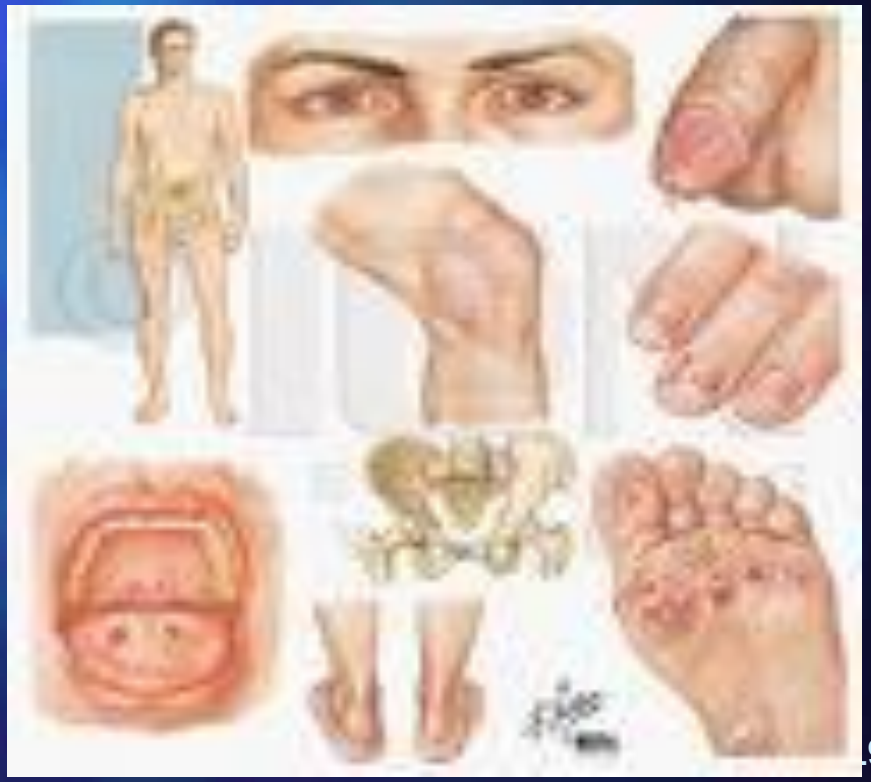
Figure 15:
Right Ectopic Pregnancy (DP= Douglas Pouch)

Men & Women

- **Reiter´s syndrome**

  - inflammation of eyes and joints, rash on genitals and soles

- **Appendicitis**



Chlamydia trachomatis

- Most commonly sexually transmitted bacterial pathogen in U.S.
  - Only HPV is a more commonly sexually transmitted disease
  - Adult males
    - Non-gonococcal urethritis (NGU)
    - Epididymitis and prostatitis

Chlamydia trachomatis (cont'd)

- Adult females
  - Urethritis, follicular cervicitis, endometritis, proctitis, salpingitis, PID and perihepatitis
- Major cause of sterility in U.S.
- May be transmitted to newborns during delivery

Chlamydia trachomatis (cont'd)

- **Laboratory Diagnosis**
  - Direct microscopic examination to find EBs
  - Cell culture
  - Enzyme immunoassay
  - Nucleic acid probes with and without amplification (PCR)
  - Serologic (antibody) assay

Ecology

- Chlamydia form two main ecological groups.
- **Infect only humans**
  - **Subgroup A**
  - **trachoma, inclusion conjunctivitis, and lymphogranuloma venereum**
- Zoonotic Infections
  - Subgroup B
  - Respiratory tract infections

C trachomatis

Trachoma

conjunctivitis

proctitis

urethritis

salpingitis

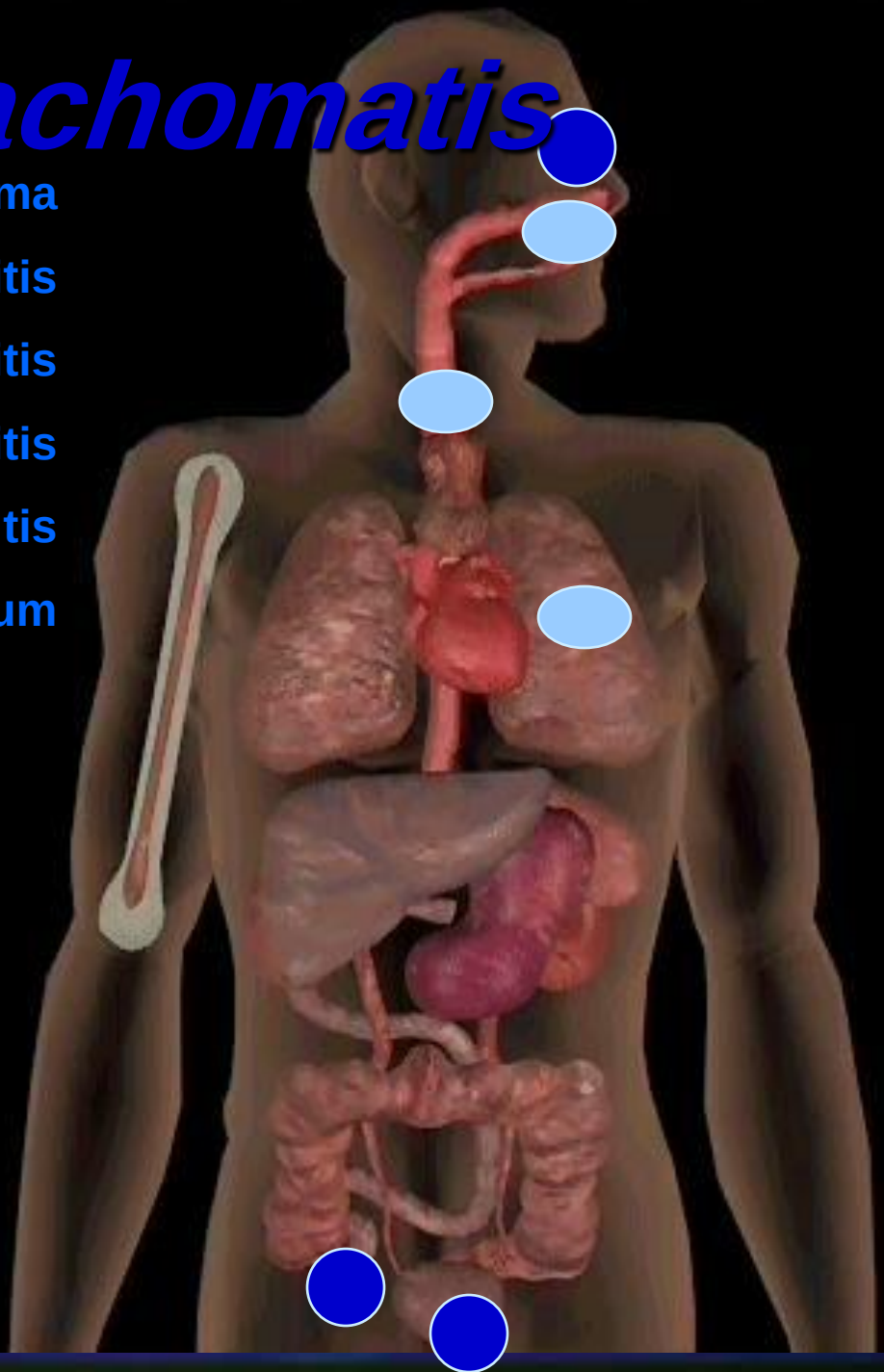
Lymphogranuloma venereum

C psittaci & C pneumoniae

Upper respiratory infection

Bronchitis

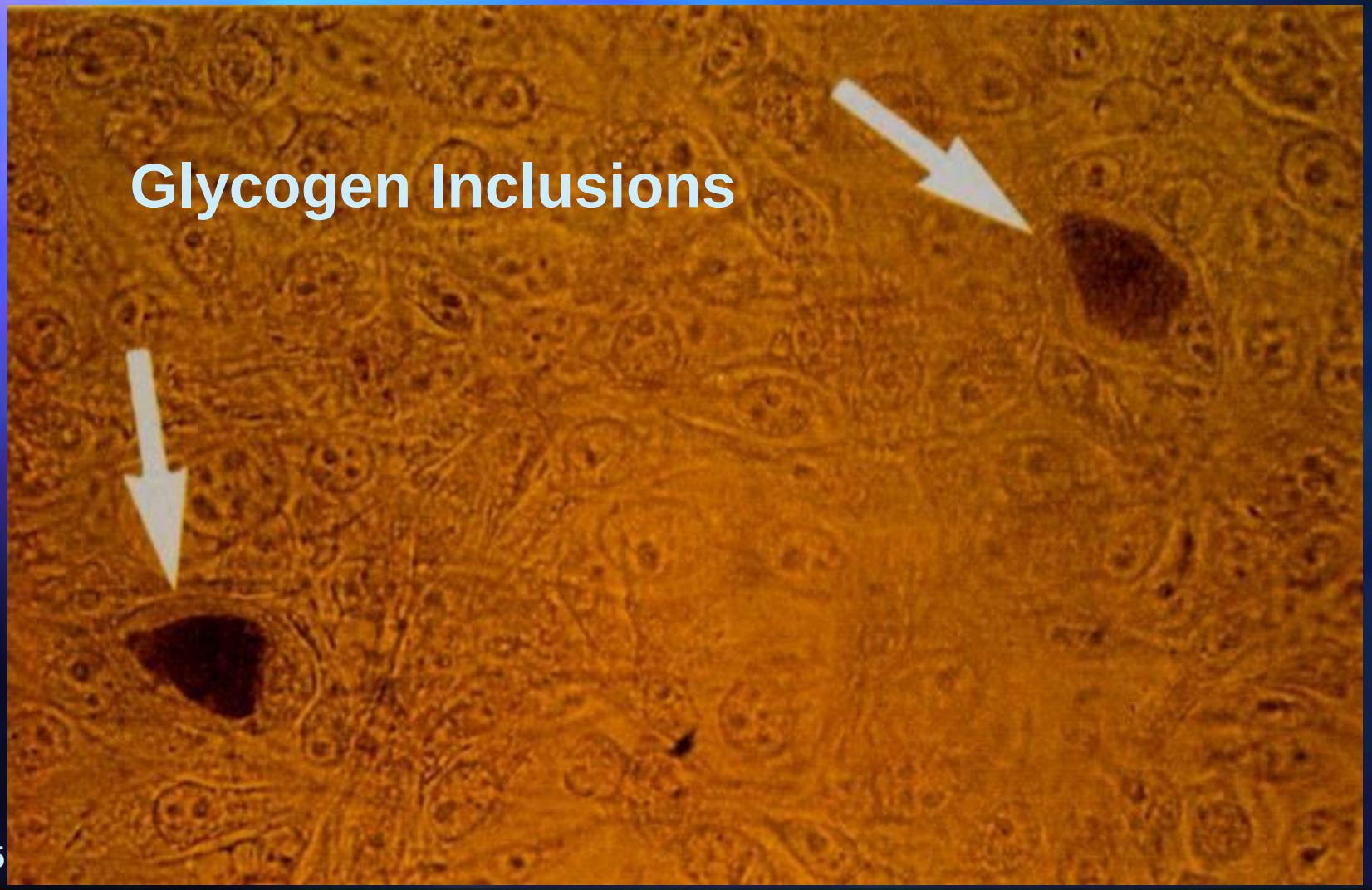
Pneumonia



Chlamydial Morphologies

- Elementary body
  - 0.25 - 0.3 um diameter
  - electron-dense nucleoid
  - Released from ruptured infected cells. Human to human
  - & bird to human.
- Reticulate Body
  - Intracytoplasmic form 0.5 - 1.0 um
  - Replication and growth. (Inclusion body)
  - without a dense center.

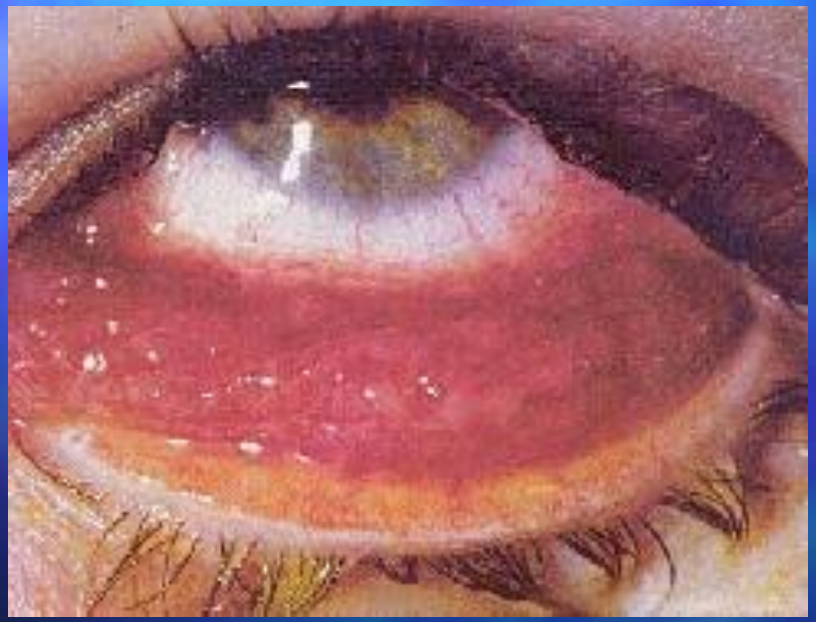
C trachomatis inclusions



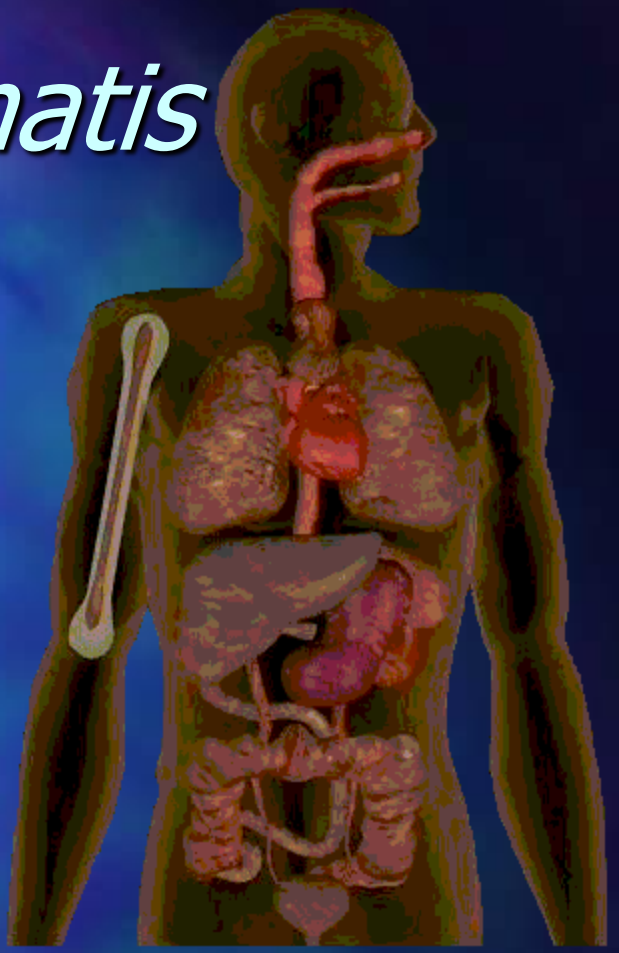
Conjunctivitis

- Inclusion conjunctivitis:
  - Transmitted by infectious secretions of the genitourinary tract
  - autoinoculation
- Infantile conjunctivitis:
  - Acquired in the birth canal -- 5-12 days after birth
  - most common type of conjunctivitis
- Antibiotic prophylaxis: erythromycin, tetracycline.

Chlamydial Infection of Ocular Conjunctiva



Chlamydia trachomatis
Clinical disease



- lymphogranuloma venereum
- nongonoccal urethritis (NGU)
- epididymitis
- salpingitis
- mucopurulent cervicitis
- pelvic inflammatory disease (PID)
- Reiter's syndrome
- neonatal chlamydia

Chlamydia Symptoms In Men

- Symptoms usually appear between 7 and 28 days after infection, usually with mild burning when urinating, a more frequent need to urinate, and a white discharge from the penis. Occasionally, blood may appear in the urine. The symptoms occur most frequently in the morning.

Nongonococcal urethritis (NGU) - Reiter's syndrome

- Swollen, painful right knee in which needle aspiration for synovial fluid was performed (yellow discoloration from the betadine prep)

Hyperlink to original site

Lymphogranuloma venereum LGV

- 200 reported cases per year.
- Incubation period is 5 to 20 days.
- Lesion: Transient vesicles on penis or vagina that are often unnoticed and patients do not usually seek medical advice.

Chlamydia pneumoniae

- This bacterium was first recognized in 1983 as a respiratory pathogen, after isolation from a college student with pharyngitis.
- Pneumonia or bronchitis, gradual onset of cough with little or no fever. Less common presentations are pharyngitis, laryngitis, and sinusitis.

Incidence

- Each year an estimated 50,000 adults are hospitalized with pneumonia in the United States. The overall incidence is unknown.

Diagnosis of chlamydia

- urine sample
- swab taken from vagina
- swab taken from opening of the urethra at the tip of the penis

Transmission

- Person-to-person transmission by respiratory secretions.
- Risk Groups
- All ages at risk but most common in school-age children. By age 20 years, 50% of population have evidence of past infection. Reinfection throughout life appears to be common.

Treatment

- short course of antibiotics: azithromycin, doxycycline or erythromycin
- one-time dose taken daily or multiple times a day for 5-10 days
- resolves within one to two weeks
- sexual abstinence during that period

Can chlamydia be cured?

Yes, chlamydia can be cured with the right treatment. It is important that you take all of the medication your doctor prescribes to cure your infection. When taken properly it will stop the infection and could decrease your chances of having complications later on.

Repeat infection with chlamydia is common. You should be tested again about three months after you are treated, even if your sex partner(s) was treated.

- **What happens if I don't get treated?**
- If you are a woman, untreated chlamydia can spread to your uterus and fallopian tubes (tubes that carry fertilized eggs from the ovaries to the uterus). This can cause pelvic inflammatory disease (PID). PID often has no symptoms, however some women may have abdominal and pelvic pain. Even if it doesn't cause symptoms initially, PID can cause permanent damage to your reproductive system.
-
- PID can lead to long-term pelvic pain, inability to get pregnant, and potentially deadly ectopic pregnancy(pregnancy outside the uterus).

- Men rarely have health problems linked to chlamydia. Infection sometimes spreads to the tube that carries sperm from the
- testicles, causing pain and fever.
- Rarely, chlamydia can prevent a man from being able to have children.
- Untreated chlamydia may also increase your chances of getting or giving HIV – the virus that causes AIDS.

Laboratory Diagnosis

- Isolate the organism from infected tissue.
  - Inoculate the yolk sac of seven-day chick embryos
  - Inoculate McCoy human cells.
- Characteristic cytoplasmic inclusion bodies in infected cells.

Immunofluorecent tests

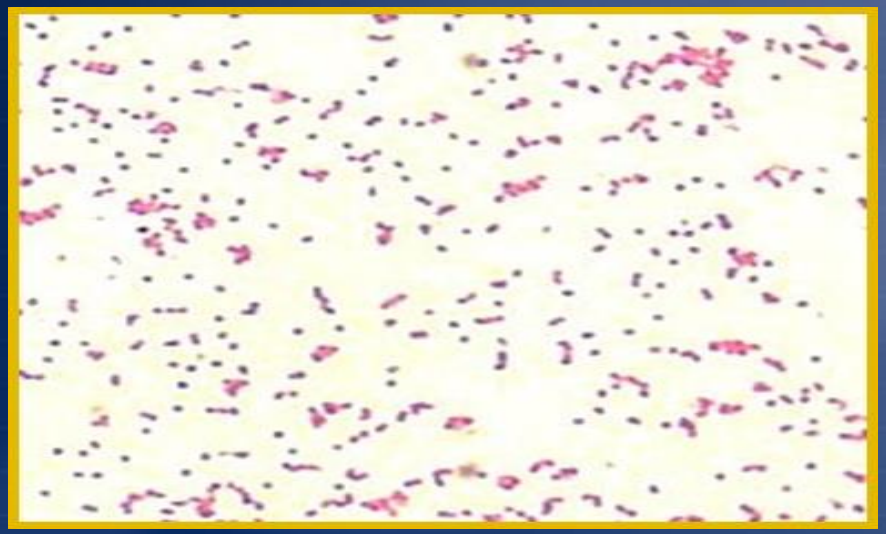
- Microimmunofluorescent tests
  - patients with eye infections
  - Check tears for the presence of anti-chlamydia antibody.
- Direct immunofluorescence
  - of conjunctive cells with fluorescein - conjugated monoclonal antibody is sensitive and specific.
  - In neonatal conjunctivitis and early trachoma

Summery

- Chlamydiae are small organisms that multiply in the cytoplasm of their host cells using unique biphasic developmental cycles.
- The EB is the infectious particle that is environmentally Stable.
- The RB is the metabolically Active Form that Divides by binary fission within a membrane-bound vacuole.
- There are three species of Chlamydia that cause disease in humans: *C trachomatis, C pneumoniae*, and *C psittaci*.
- C trachomatis is responsible for sexually transmitted diseases that include cervicitis, pelvic inflammatory disease, urethritis, epididymitis, LGV, and proctitis, and when transmitted to infants of infected pregnant women, infant inclusion conjunctivitis and eosinophilic pneumonia.

- Treatment of infections caused by *C trachomatis* requires doxycycline or azithromycin.
- *C pneumoniae* causes a variety of upper and lower respiratory infections. Pharyngitis is common, and atypical pneumonia resembling that of M pneumoniae is responsible for 5–15% cases of community-acquired pneumonia.

Brucellosis



Presented by:

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The Many Names of Brucellosis

Undulant Fever,

Malta Fever,

Mediterranean Fever,

Enzootic Abortion,

Epizootic Abortion,

Contagious Abortion,

Bang's Disease

BRUCELLOSIS

Brucellosis, a bacterial disease caused by members of the genus Brucella, remains one of the most common zoonotic diseases worldwide, also cause abortion which is common and rate varies from 30%-80%. So this disease is very dangerous as it can affect man and also cause large economic losses by decreasing milk yield and aborted fetus.

Points of interest

- Define
- Causative agent
- Route of infection
- Pathogenesis
- Microscopic examination
- Macroscopic examination

Brucellosis

Define

It is a chronic bacterial infection affect domestic animals and man. it is characterized by abortion.

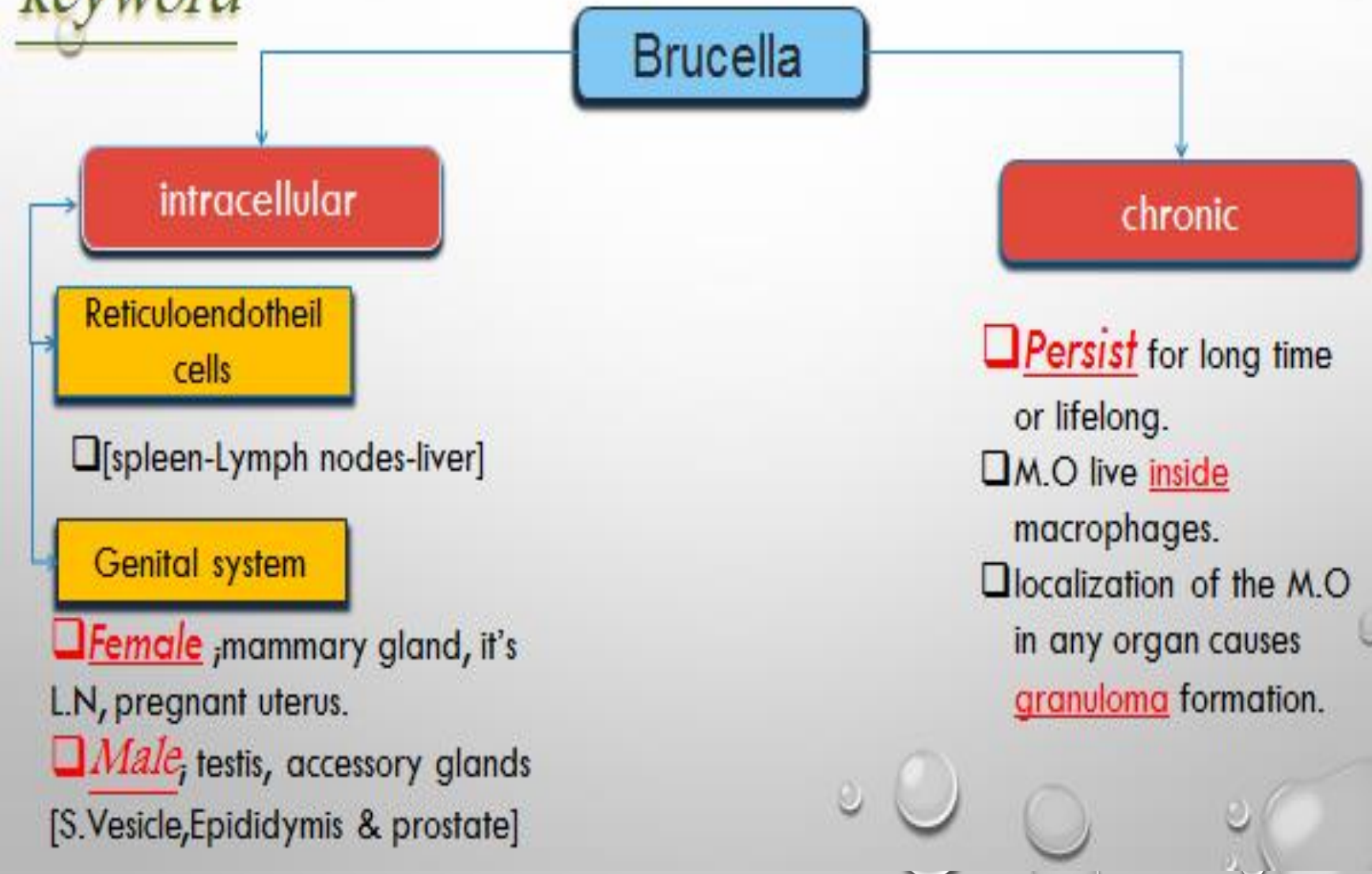
Cause

Brucella species, which is gram –ve bacilli and intracellular.

B.melitensis	*B.abortus*	*B.suis*	*B.ovis*
↓	↓	↓	↓
Goat	cattle	pig	sheep

MIND MAPPING OF BRUCELLOSIS

keyword



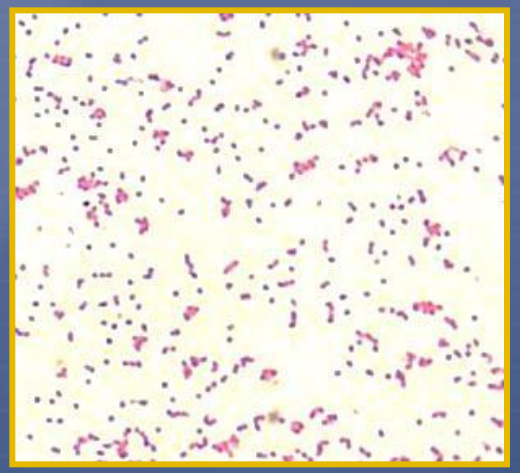
ROUTE OF INFECTION

- Ingestion of raw milk
- Through conjunctiva
- Intact or broken skin

Humans are generally infected in one of three ways: eating or drinking something that is contaminated with the bacteria, breathing in the presence of organisms (inhalation), or having the bacteria enter the body through skin abrasions. Inhalation of Brucella organisms is not the common route of infection, **but** it can cause significant hazard for people in certain occupations.

THE ORGANISM

Brucella spp.



- Gram negative coccobacillus
  - Facultative, intracellular organism
- Multiple species
  - Associated with certain hosts
- Environmental persistence
  - Withstands drying
  - Temperature, pH, humidity
  - Frozen and aborted materials, dust, soil

- It affects people of all age groups and of both sexes.
- The genus *Brucella* consist of eight classical species,

Human Brucellosis & Associated Species

Species	Animal Reservoir	Clinical Disease
Brucella melitensis	Goats, sheep	Severe acute disease with complications (common)
Brucella abortus	Cattle	Mild disease with suppurative complications (uncommon)
Brucella suis	Swine	Severe Chronic, suppurative, destructive disease
Brucella canis	Dogs	Mild disease with suppurative complications (uncommon)

REVIEW

Brucellosis in humans is predominantly caused by four different species of Brucella:
Brucella melitensis, Brucella suis, Brucella abortus and Brucella canis.
Though all of these species can cause human brucellosis, Brucella melitensis is the most prevalent worldwide, and is known as main causative agent of human brucellosis.

HISTORY

History of Brucellosis

Professor FEG Cox. *The Wellcome Trust, Illustrated History of Tropical Diseases*

- Sir David Bruce (1855-1931)
  - British Army physician and microbiologist
  - Discovered *Micrococcus melitensis*

The mode of infection is:

on contact *Brucellae* penetrate the skin or mucosal membranes and enter the lymph nodes, which become hemorrhagic, resulting in bacteremia, which facilitates dissemination throughout the body. During the early phase of infection, *Brucellae* invade macrophages, adapt to the acidic enviornment, and multiply in the vacuolar compartments; it prevents phagosome/lysosome fusion. Brucellosis is a systemic infection that can involve any organ or organ system of the body.

PATHOGENESIS

Bruce lla

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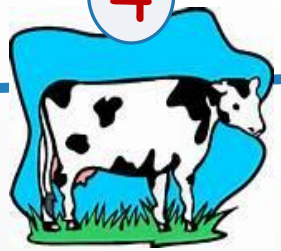


Regional L.N

Acute lymphadenitis

L.N is enlarged, hyperplastic ,sinuses are filled w' NQ and plasma cells.

Th.d uct

bloo d

bactere mia

Localization in organs

others

Synovi al structu res

Rich in erythri tol

- Arthritis
- Purulent tendovagin itis

- Help growth of M.O

Genital system

Femal e

male

Reticloendot heil cells

Liver-spleen-L.N

M.O → Muliplicate → epitheloi d cells

N.B wherever the org.is localized, a granuloma develops?

- L.O and plasma cells accumulated around these

(continue) pathogenesis

Localization in genital organs

- **Female**
  - Mammary gland
    - ***Chronic mastitis***
  - Pregnant uterus
    - Intracellular edema + necrosis
    - Carancle cotyledon+chorionic villi
    - ***Abortion***
- **Fetus**
  - placenta
    - ***Retained placenta***
  - Respiratory system
    - ***Broncho-pneumonia***
- **male**
  - Testis
    - ***orchitis***
  - Accessory gland (Seminal.vesc + prostate)
    - ***Seminal-vesiclitis***
    - ***prostitis***

Symptoms:

are non specific, which may include fever, chills, headache, pain, fatigue, dementia, and arthritis, generally occurring within 2-3 weeks of inoculation.

The **complication** involves osteoarticular complication, gastro intestinal complications, genitourinary complications, neurological complications, cardiovascular complications.

Brucellosis in Humans

- **Human brucellosis = Bang's disease**, named for Bernhard Bang & Sir David Bruce who discovered *Brucella*
- **Facultative intracellular pathogens** of mononuclear-phagocyte system (formerly reticuloendothelial system which is involved in immune defense against microbial infection and removal of worn-out blood cells)
  - Bacteria are phagocytosed by macrophage or polymorphonuclear leukocyte
  - Survive intracellularly by inhibiting killing
  - Carried to spleen, liver, bone marrow, lymph nodes, kidneys
- **Form granulomas** (mass of granulation tissue produced in response to chronic infections, inflammation, or foreign bodies) and cause **destructive tissue damage**

Clinical Presentation of Human Brucellosis

- **Acute disease** often develops with initial nonspecific symptoms of malaise, chills, fatigue, weakness, myalgias (muscles), weight loss, arthralgias (joint), and nonproductive cough
- **Mild disease** with rare suppurative complications
- **Chronic disease and recurrence** are common because it can survive in phagocytic cells and multiply to high concentrations
- May also take the form of **destructive lesions**

DIAGNOSTIC TOOLS FOR BRUCELLOSIS

1. **Culture**: Blood culture provides definite proof of brucellosis but may not provide a positive result for all patients.
2. **Antigen detection:** Antigen detection methods by enzyme linked immunosorbent assay (ELISA) are potentially useful and recently several antigen detection systems are under development.

3. **Genome detection:** Polymerase chain reaction (PCR) has been explored for the rapid detection and confirmation of *Brucella*. Molecular characterization techniques are very useful tools for differentiating *Brucella* spp.,

4. **Antibody detection:** The limitations of above mentioned tools make serology, directed against antibody detection, the most useful tool. Antibodies usually begin to appear in the blood at the end of the first week of the infection, IgM appearing first followed by IgG.

(A) Agglutination tests

Rose Bengal Plate Test (RBPT) is one of a group of tests known as the buffered Brucella antigen tests which rely on the principle that the ability of IgM antibodies to bind to antigen is markedly reduced at a low pH . It is performed on glass slide with coloured bacterial antigen, and this test is of value as a screening test especially in high risk rural areas where it is not possible to perform SAT. Whenever possible, a serum that gives a positive result should be confirmed by a more specific test.

RBPT also plays a great role in the rapid confirmation of neurobrucellosis, arthritis, epididymo-orchitis, and hydrocele due to Brucella. The test is an excellent screening test

Serum Agglutination Test (SAT) remains the most popular and worldwide used diagnostic tool.

SAT measures the total quantity of agglutinating antibodies (IgM and IgG) and the quantity of specific IgG is determined by 2-mercaptoethanol (2ME). The type of antibody is important, as IgG antibodies are considered a better indicator of active infection and the rapid fall in the level of IgG antibodies is said to be prognostic of successful therapy. This test is simple and cheap to perform but its lack of sensitivity and specificity mean that it should only be used in the absence of alternative techniques.

Coombs test

Complement fixation test (CFT) has good sensitivity and specificity

(B) ELISA

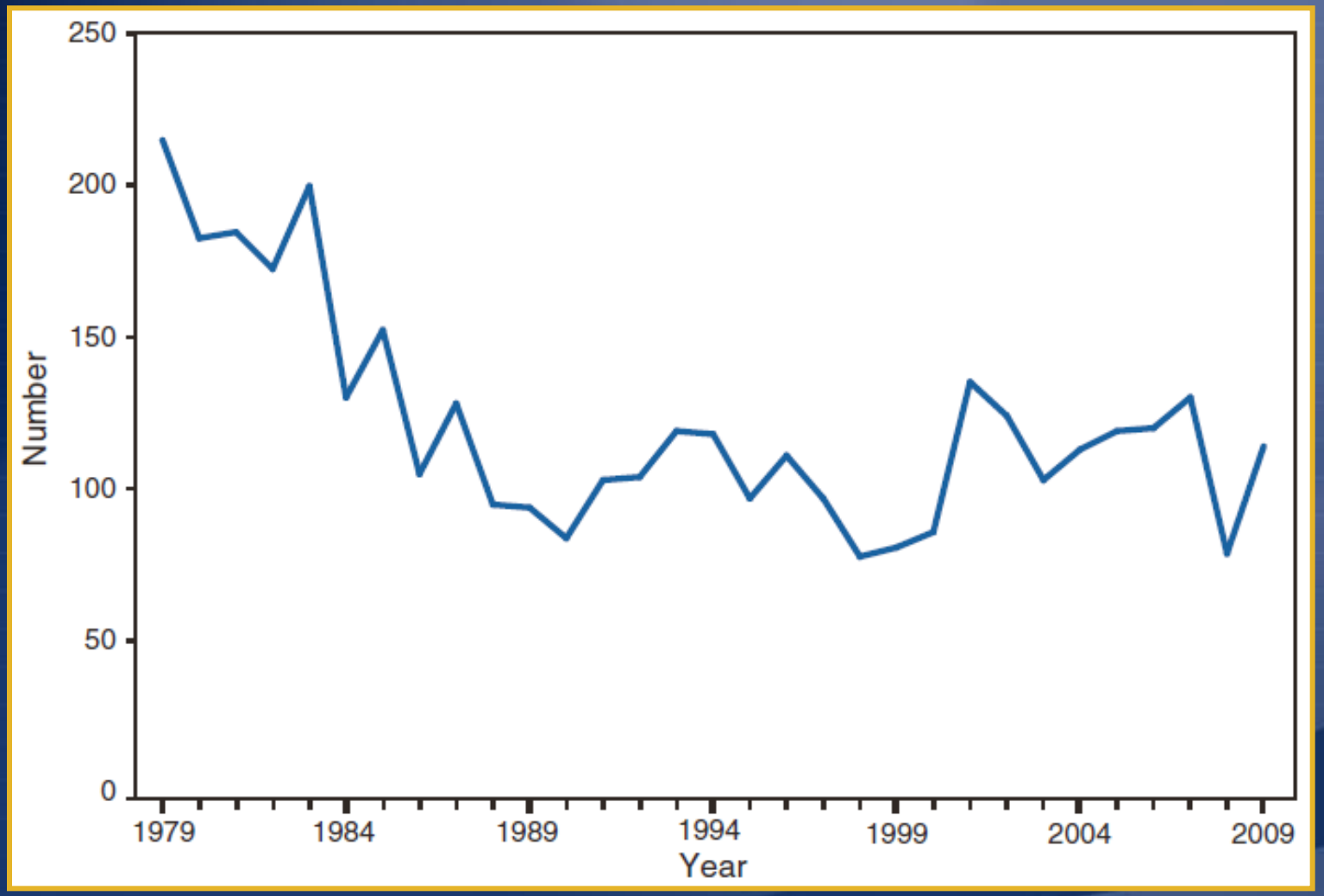
The ELISA tests offer excellent sensitivity and specificity whilst being robust, fairly simple to perform with a minimum of equipment and readily available from a number of commercial sources in kit form.,

EPIDEMIOLOGY

Populations at Risk

- Occupational disease
  - Cattle ranchers/dairy farmers
  - Veterinarians
  - Abattoir workers
  - Meat inspectors
  - Lab workers
- Hunters
- Travelers
- Consumers
  - Unpasteurized dairy products

Brucellosis: Reported cases, by year
United States, 1979 – 2009



DISEASE IN HUMANS

Disease in Humans

- Incubation period
  - Variable; 5 days to three months
- Multisystemic
  - Any organ or organ system
  - Cyclical fever
- Flu-like illness
  - Chronic illness possible

Complications of Brucellosis

- Most common
  - Arthritis, spondylitis, epididymo-orchitis, chronic fatigue
- Neurological
  - 5% of cases
- Other
  - Ocular, cardiovascular, additional organs and tissues

Congenital Brucellosis

- Variable symptoms
  - Premature delivery
  - Low birth weight
  - Fever
  - Jaundice
  - Hepatomegaly
  - Splenomegaly
- Abortion risk unclear

Vaccination

- Vaccines against *Brucellae* have varying degrees of success in controlling the disease in animals; however, human vaccines are **not currently available** and the animal vaccines currently in use are pathogenic to humans.
- Both humoral and cell-mediated immune responses develop in brucellosis patients, but the cellular immunity is the essential component.

PREVENTION AND CONTROL

PREVENTION

The prevention of human brucellosis is based on Education to avoid consuming unpasteurized milk and milk derivatives. Barrier precautions for hunters and professionals at risk (butchers, farmers, slaughterers, veterinarians). Careful handling and disposal of afterbirths, especially in cases of abortion. Serological or other testing of animals; immunization of herds/flocks may be envisaged; eliminate infected herds/flocks. Occupational hygiene and food hygiene; Vaccination is not generally recommended. All dairy products should be prepared from heat-treated milk; Consumption of raw milk or products made from raw milk should be avoided. Meat should be adequately cooked. Special precautions should be taken by laboratory workers; Physicians and health workers should be aware of the possibility of brucellosis. Public health education should emphasize food hygiene and occupational hygiene.

TREATMENT

- Treatment for brucellosis aims to relieve symptoms, prevent a relapse of the disease and avoid complications. You'll need to take antibiotics for at least six weeks, and your symptoms may not go away completely for several months.
- The disease can also return and may become chronic

- Rifampicin is active in vitro against Brucella species, is remarkably lipid soluble, and it accumulates within eukaryotic cells. In order to provide a completely oral regimen with which to treat brucellosis, the combination of doxycycline plus rifampicin, with both drugs administered for six weeks, was recommended by the WHO in 1986.
- Tetracycline administered for at least six weeks has long been the standard treatment of human brucellosis
- For infants, tetracycline is toxic, so children are treated with trimethoprim-sulfamethoxazole

CONCLUSION

Brucellosis has been eradicated from various developed countries but still it remains an important veterinary public health problem in most of the developing world as abortions and infertility in herds result in severe economic loss. Human brucellosis is commonly reported among laboratory workers, slaughter house employees, farmers and veterinarians who may be exposed to infected animals. Due to its heterogeneity and poorly specific clinical symptoms, the diagnosis of brucellosis always required laboratory conformation either by isolation of pathogen or by demonstration of specific antibody. The serological test available for diagnosis of brucellosis remains most useful test for preliminary identification of the disease besides its limitation of low sensitivity. Therefore, there is a need to develop rapid, reliable and user friendly system for disease diagnosis and alternative vaccines approaches. Because of inherent problems with bacterial isolation, inefficiency, cost, danger and other factors,

most laboratories prefer to use other, more cost effective methods. Molecular biology as a diagnostic tool is advancing and will soon be at the point of replacing actual bacterial isolation. It is rapid, safe and cost effective, the only real problems being some uncertainties regarding specificity.

Parvobacteria

Haemophilus, *Brucella* and *Bordetella*

Presented by:

Shaymaa H. Al-Kubaisy

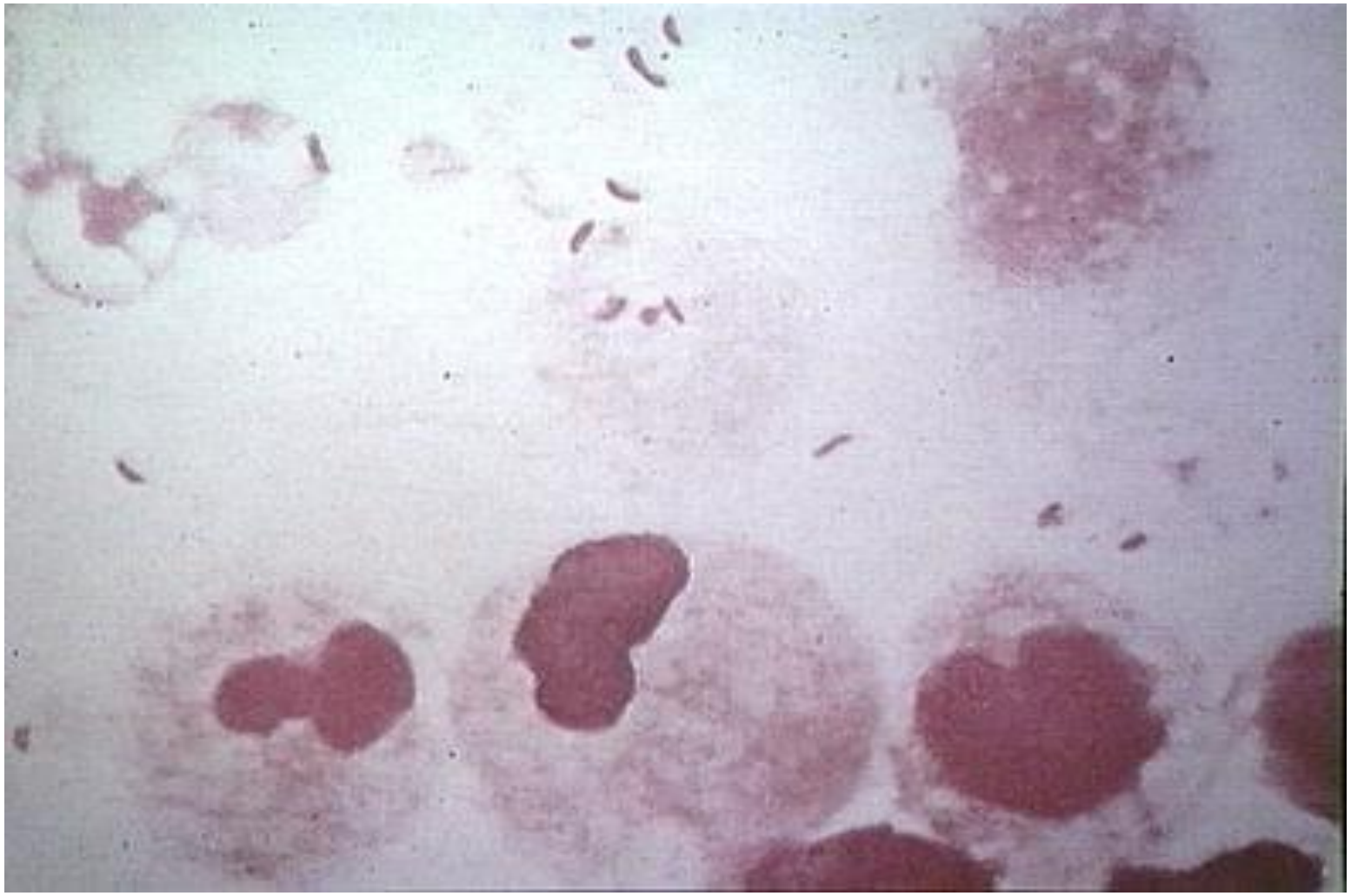
B.Sc. M. Ph. D. Med. Microbiology

Parvobacteria

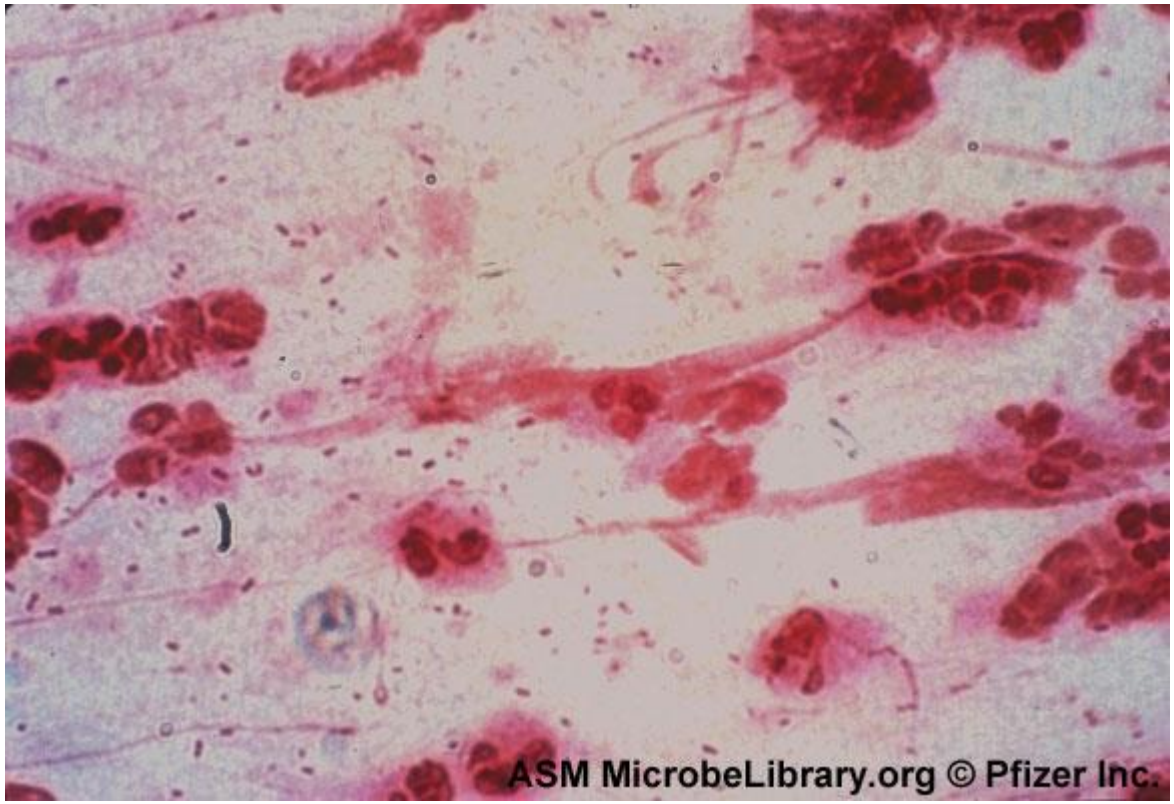
Haemophilus influenzae	Invasive disease - meningitis, bacteraemia, osteomyelitis Non-invasive disease –respiratory tract.
Bordetella pertussis	Whooping Cough
Brucella spp.	Brucellosis
***Yersinia* spp.**	Diarrhoea and systemic disease
Pasteurella	Wound infection after dog/cat bites
Francisella	Tularaemia
Legionella	Pneumonia

H. influenzae

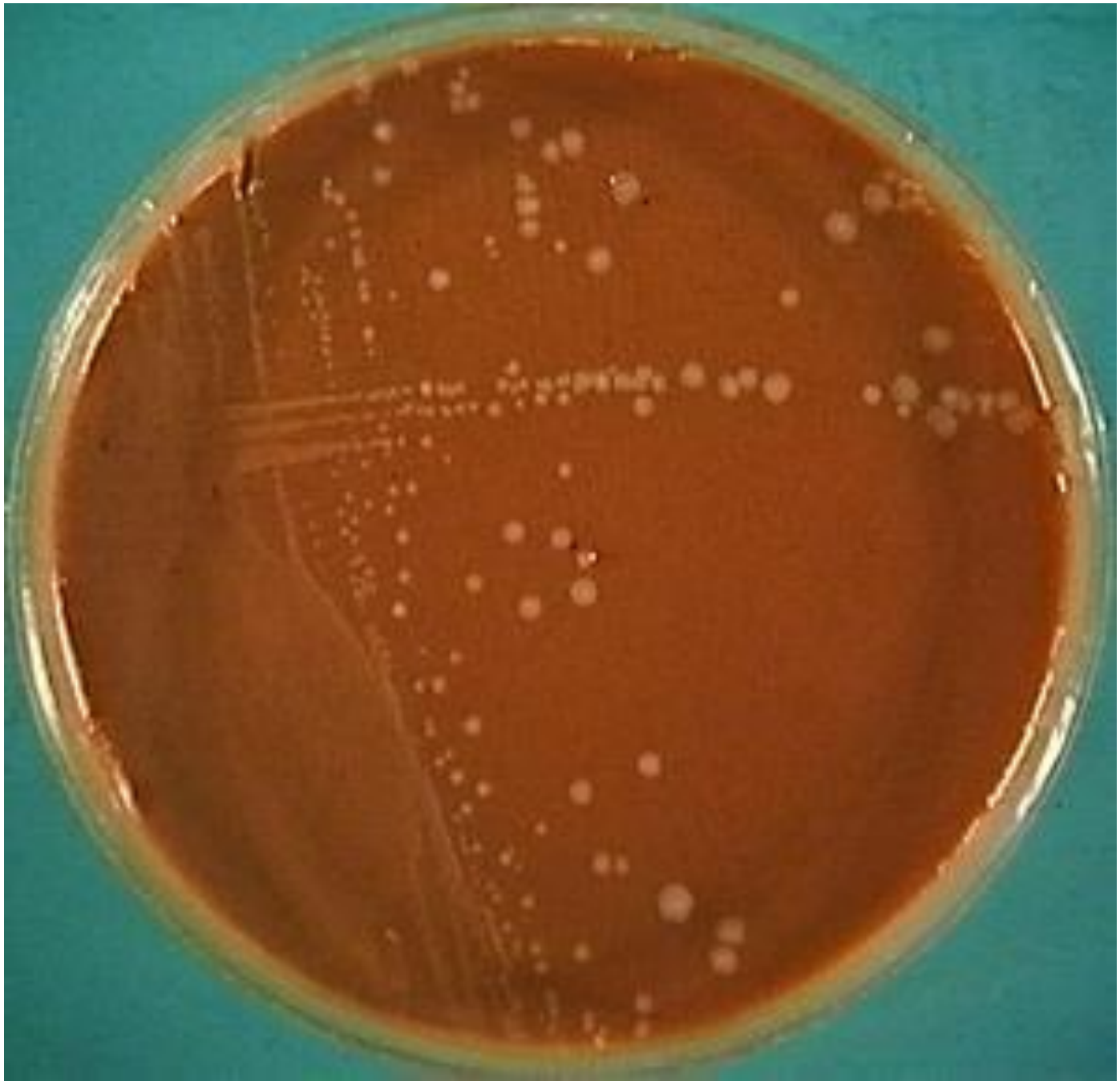
- Small, non-sporing, non-motile bacterium
- Encapsulated strains isolated from cerebrospinal fluid are gram-negative coccobacilli
- Non encapsulated organisms from sputum are pleomorphic
- Requires preformed growth factors that are present in blood, specifically
  - X factor (i.e., hemin – from iron containing pigments)
  - V factor (NAD or NADP).
- Usually grown on chocolate blood agar

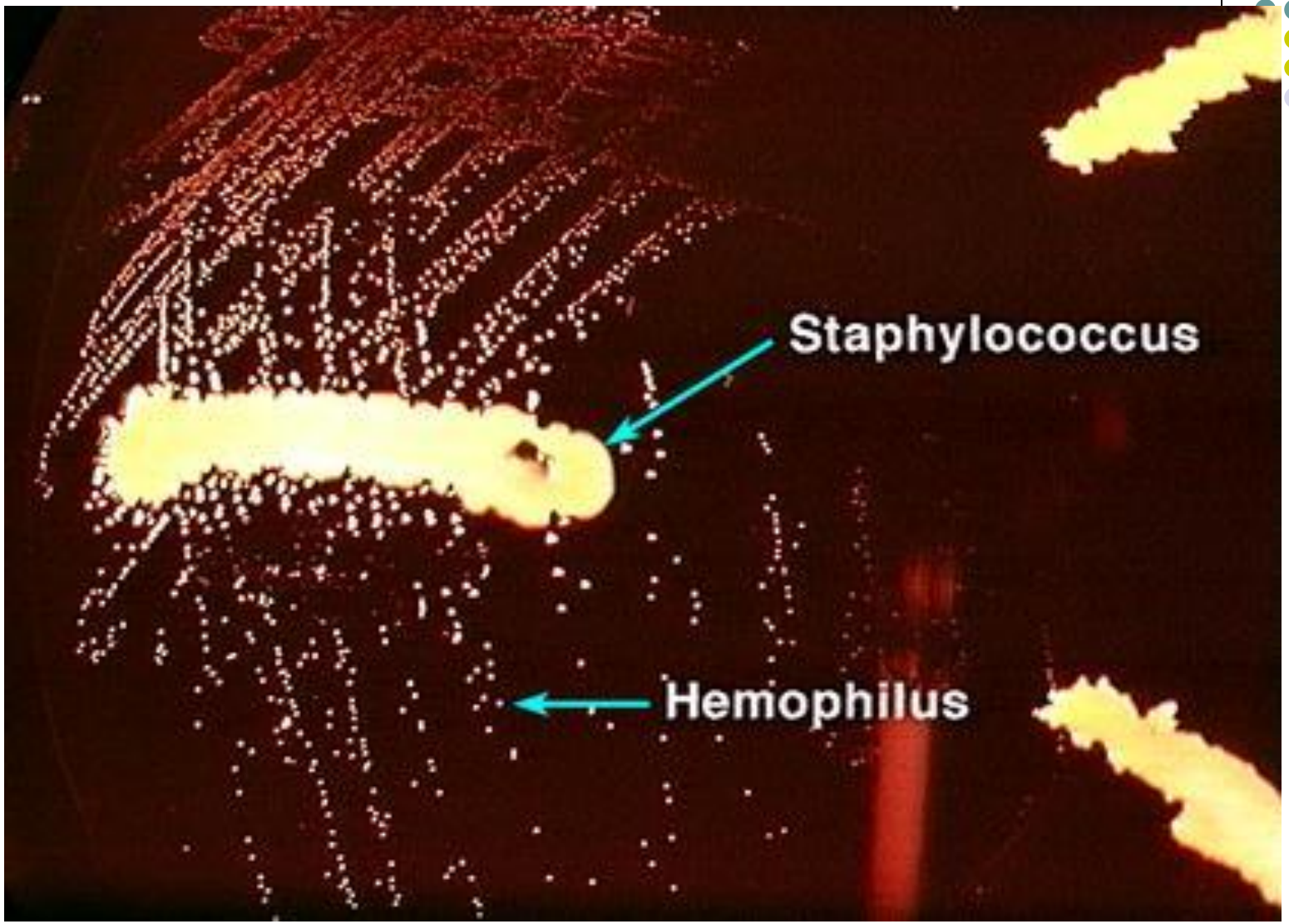


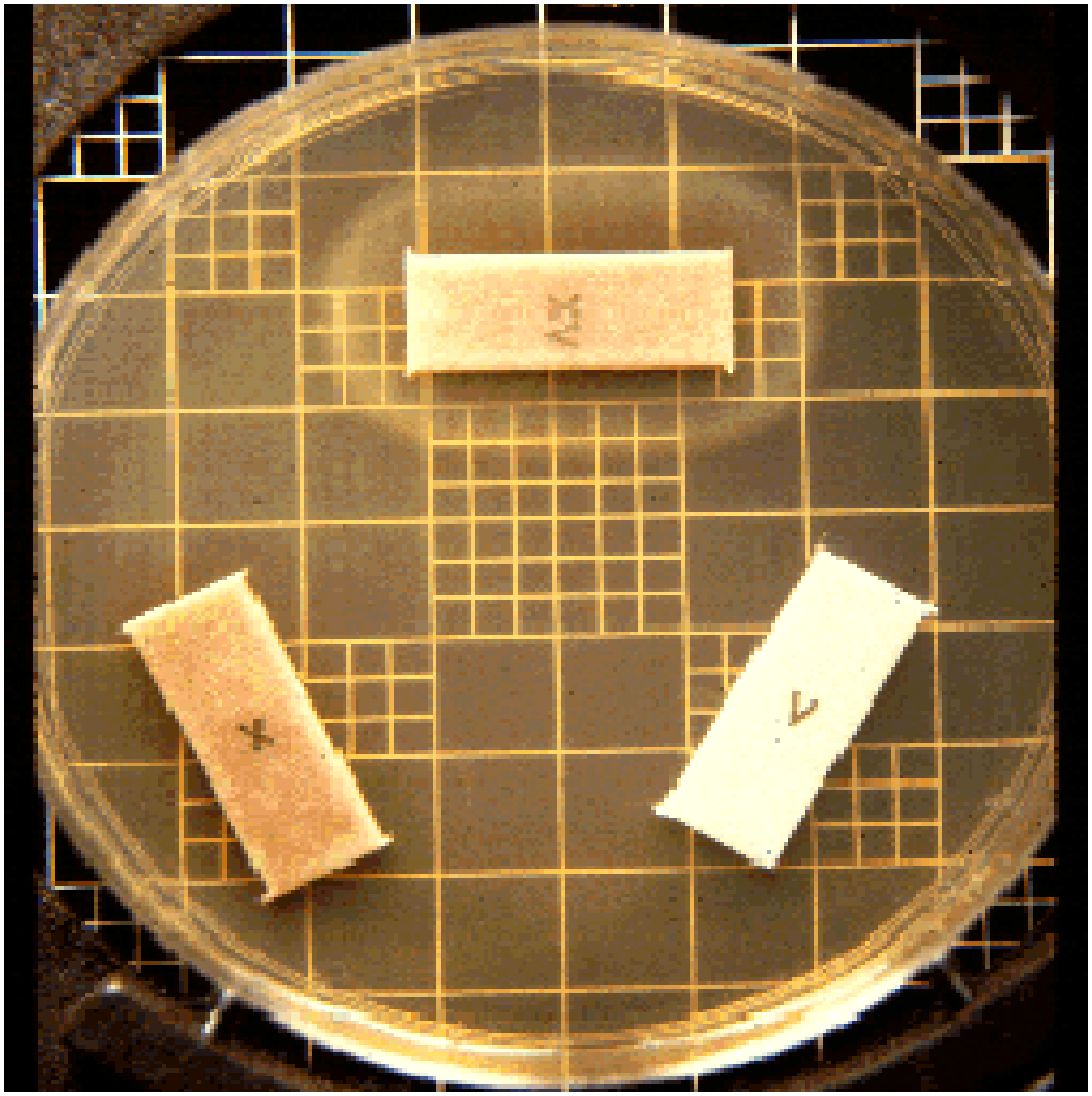
Gram stain of *H. influenzae* from a CSF



Gram stain of *H. influenzae* from sputum




Staphylococcus
Hemophilus



Haemophilus influenzae /nutritional factors
X=hemin V= NAD

Haemophilus influenzae **requires X and V factors for growth. In this culture haemophilus has only grown around the paper disc that has been impregnated with X and V factors. There is no bacterial growth around the discs that only contain either X or V factor**

Laboratory Diagnosis

- Gram stain /Gram negative coccobacilli
- With pleomorphism- different shapes in Clinical specimens e. g. C.S.F

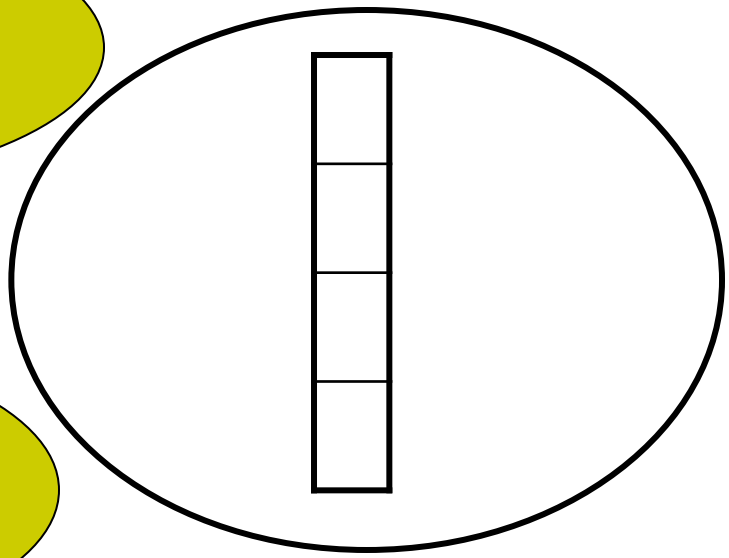
Habitat:

- Usually found in upper respiratory tract of man.
- Culture
- All species need blood or blood products for growth
- Haemophilus influenzae can on:

  e.g. (A) Chocolate agar

- e.g. **(A) Chocolate agar**
- **B) Blood agar** with growth of *Staphylococcus aureus* – a phenomenon called satellitism

Large colonies of *H. influenzae* near Staphylococcus colonies



Small colonies of *H. influenzae* away from colonies of *Staph. aureus*

Colonies of *Staphylo-coccus aureus* producing V factor

Blood Agar Plate

Providing X Factor

c) On nutrient agar with:

Factor Required	Species
X and V	*H. influenzae, H. aegyptius*
	H. haemolyticus
X	*H. ducreyi*
V	*H. parainfluenzae, parahaemolyticus*

Laboratory Diagnosis

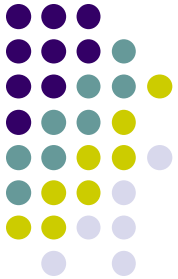
Specimens depends on the type of diseases

a. **CSF – in meningitis**
b. **Blood –meningitis and bacteriaemia and all types of invasive disease.**
c. **Sputum – in pneumonia**
d. **Swab – in cellulitis**
e. **Synovial fluid – in arthritis**

Direct smear shows pus cell + pleomorphic gram –ve coccobacilli

CSF Culture, on chocolate agar

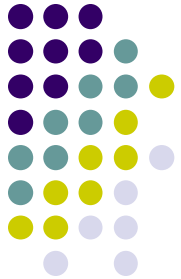
CSF detection for presence of antigens by agglutination



***catalase and oxidase tests, both of which should be positive**

H. influenzae will grow in the hemolytic zone of *Staphylococcus aureus* on blood agar plates; the hemolysis of cells by *S. aureus* releases factor V which is needed for its growth. *H. influenzae* will not grow outside the hemolytic zone of *S. aureus* due to the lack of nutrients such as factor V in these areas. Fildes agar is best for isolation.

Bordetella pertussis



- Gram-negative coccobacillus
- Nutritionally fastidious, normally cultivated on medium containing blood
- Primarily a human pathogen
- Other members of the genus *Bordetella* can cause disease in animals
- Causes Pertussis (Whooping cough)

Laboratory Diagnosis

Gram stain of
Bordetella pertussis

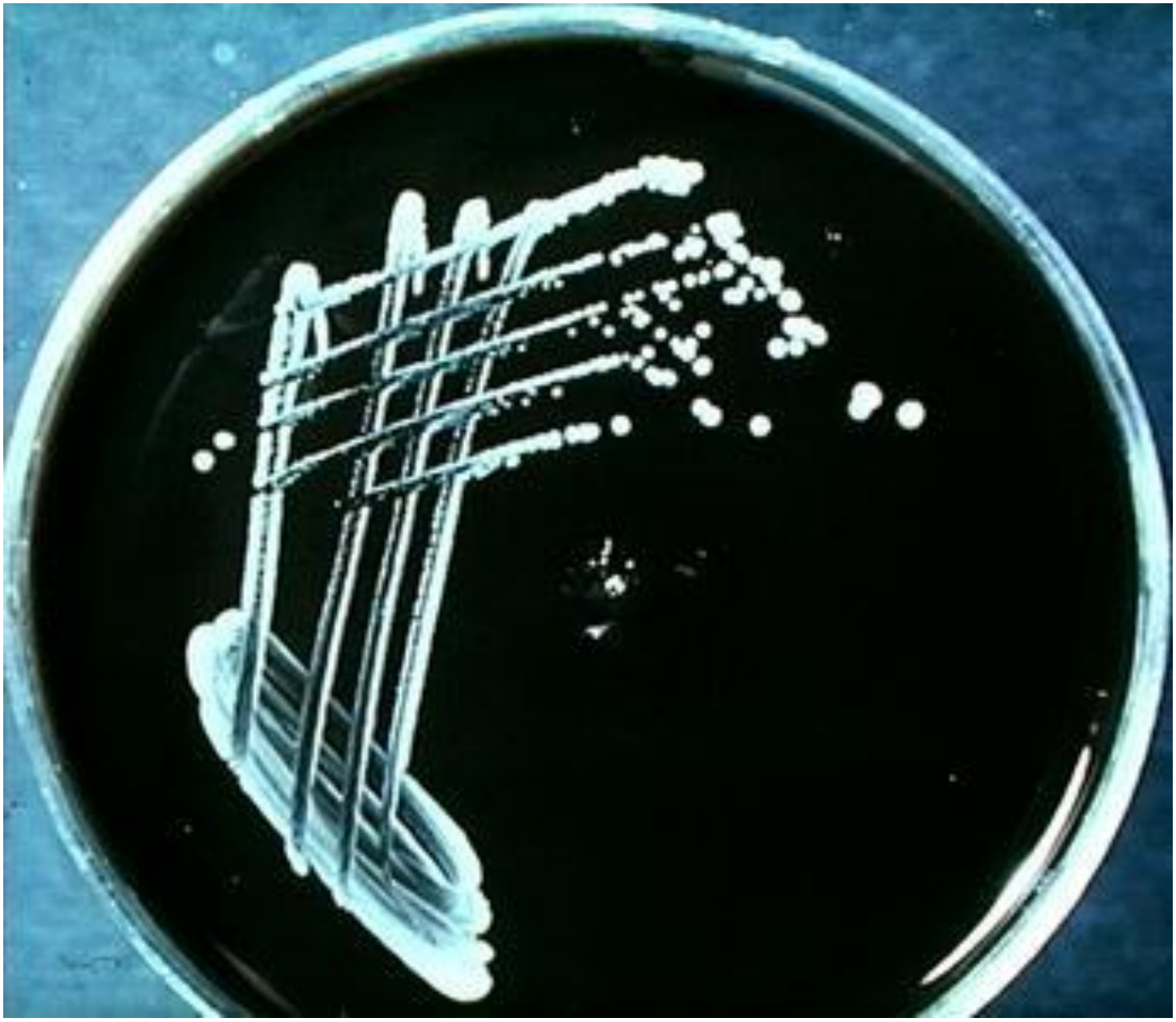
Bordetella pertussis **is a Gram-negative, aerobic coccobacillus of the genus *Bordetella*, and the causative agent of pertussis or whooping cough. The bacterium is spread by coughing and by nasal dripping. The incubation period is 7-14 days**

Cultural Characteristics :

- *Bordetella pertussis* is a slow-growing organism that requires specialized conditions for growth. It is the most fastidious species within the genus. One of the media used for its cultivation is charcoal agar supplemented with 10% horse (or sheep) blood and cephalexin. Plates are incubated in air without elevated carbon dioxide at 35˚ C for a minimum of 7 days before being reported as negative (most isolates are detected in 3 to 4 days). Colonies are small, shiny and round. With age they become whitish grey. Repeated subculture of *B.pertussis* leads to loss of fastidiousness and laboratory adaptation to a variety of media.

- **Biochemical Properties :**
- Biochemical Properties Biochemically inactive Do not ferment sugars, form indole, reduce nitrates, utilize citrate or split urea Produces oxidase & catalase
- **PCR**

- **Serology (**Swab by Immunofluorescence)
  (Rapid Method)



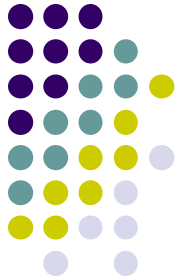
Brucella species

- Gram-negative coccobacillus
- Facultative intracellular parasites
- Six species
  - *B. abortus* - cattle
  - *B. suis* - pigs
  - *B. melitensis* - goats
  - *B. canis* - dogs
  - *B. ovis* - sheep
  - *B. neotomae* - desert wood rats
- Cause zoonoses worldwide

Common Features

Gram negative
Capsulated
Non spore forming
Non-motile
Grows on enrichment medium: Serum cholesterol
Anaerobic
Grows at 37C
Small Colonies
Brucella ovis and Brucella canis show rough growth, while others show smooth growth.
Urease test positive
Survives within macrophages (intracellular)
Mostly causes Enteritis in Rodents and Abortion in mammals.

Diagnosis

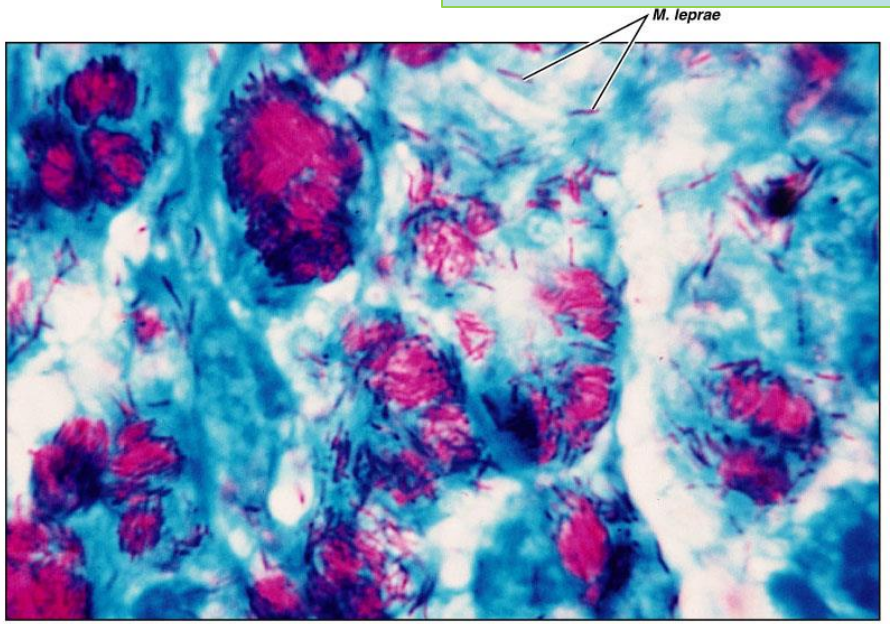


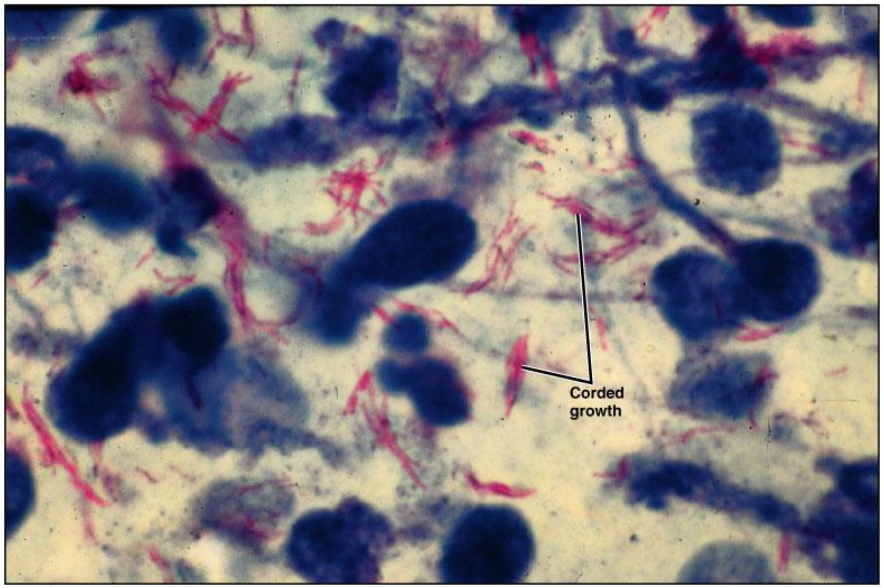
- Serologic tests
  - Serum agglutination test (SAT) or microagglutination test
    - Most widely used
    - Not useful for following treatment as titres remain high
  - ELISA
    - Most sensitive – IgG and IgM
  - Brucellacapt
    - All antibodies detected by an inmmunocapture-agglutination technique
- No single titre assay is always diagnostic
  - Consider titre of >1:160 as suspicious

Thank you

Mycobacterium species

Represented by:-
Lecturer Dr. Shaymaa H. Al-Kubaisy
B.Sc. M.Sc. Med. PH.D. Microbiol





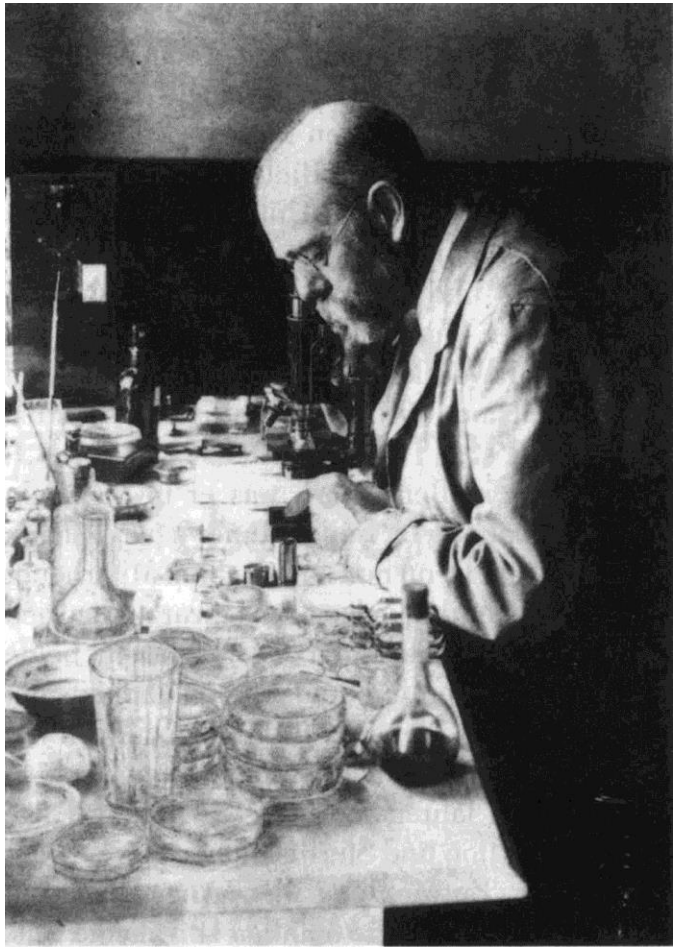
Important Human Pathogens

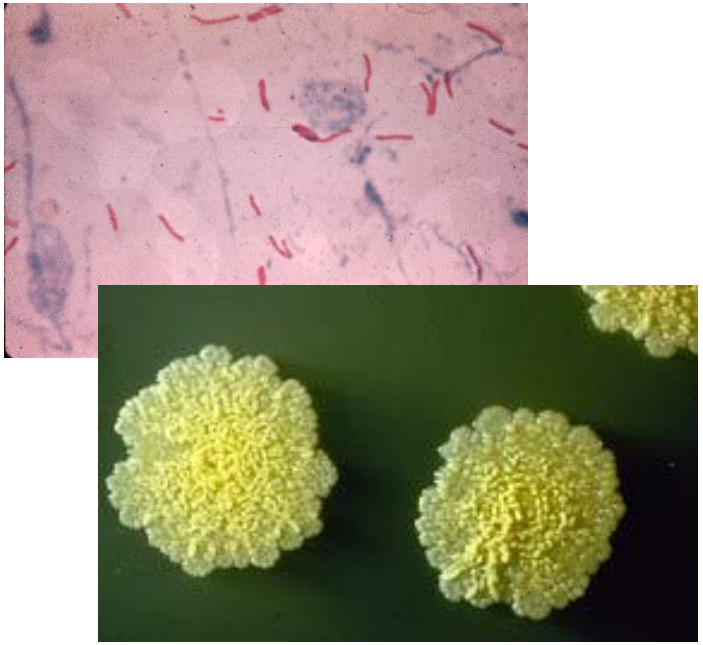
Mycobacterium tuberculosis

Mycobacterium leprae (uncommon)

Mycobacterium avium-intracellulaire Complex (MAC) or (M. avium)

M tuberculosis as causative agent for tuberculosis





Robert Koch

The pathogens include the organisms responsible for human and bovine **tuberculosis** and for **leprosy**.

It is convenient to divide mycobacteria of clinical interest into:

1-*Mycobacterium tuberculosis* complex (MTC) which includes *M. tuberculosis*, *M bovis*, BCG, *M. africanum*, and ***M. microti***. These mycobacteria associated with tuberculosis and they are always pathogenic for man and animal

2-Mycobacteria other than tuberculosis bacilli (MOTT)

which associated with human disease also these mycobacterial spp. Called **atypical**, **anonymous**, **non tuberculosis, tuberculoid bacilli.**

Diseases caused by *Mycobacterium* species

M. tuberculosis **– tuberculosis**

M. leprae **– leprosy**

M. avium **- lung and skin infections in immunocompromised hosts**

General Characteristics

- Slender, slightly curved or straight rod-shaped organisms
- Non-motile
- Do not form spores
- Cell wall with extremely high lipid content
  - Staining requires longer time or application of heat
  - Once stained, resist decolorization with acid-alcohol (acid-fast)

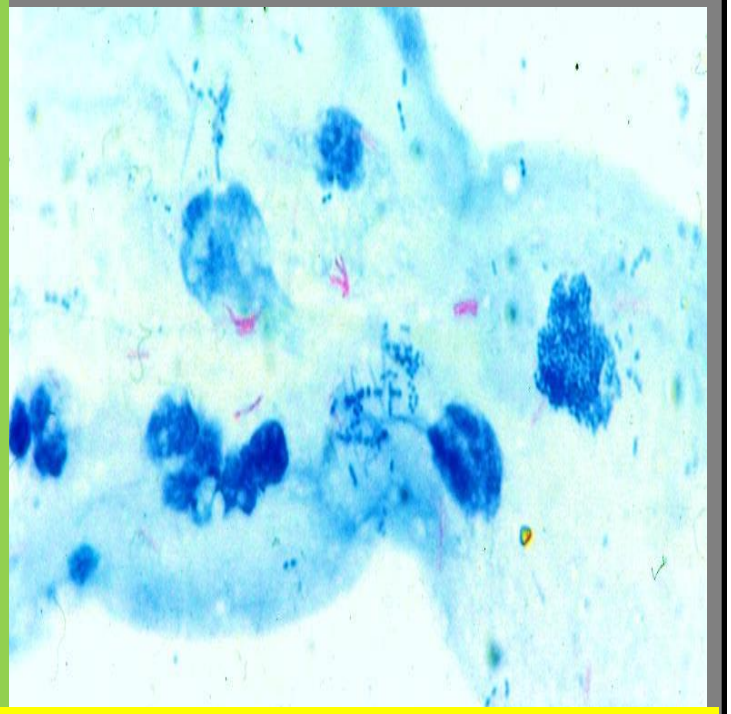
Laboratory Diagnosis

- ***Specimens: include sputum,***

- ***Early morning sputum specimens collected on 3 consecutive days, from a deep productive cough, give the best results***

Laboratory Diagnosis

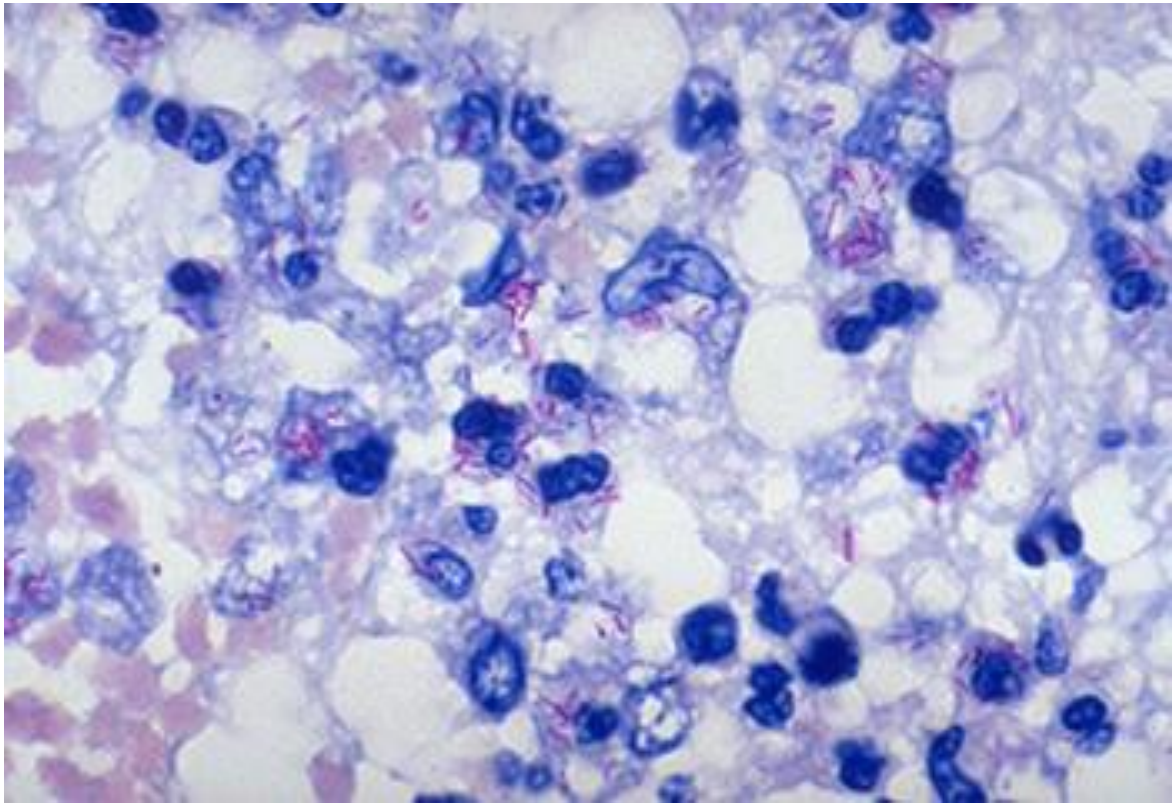
- ***Direct Detection:***
  1. *Smears: Ziehl-Neelsen (Z-N). Under the ordinary light microscope, AFB appear pink in a blue background*



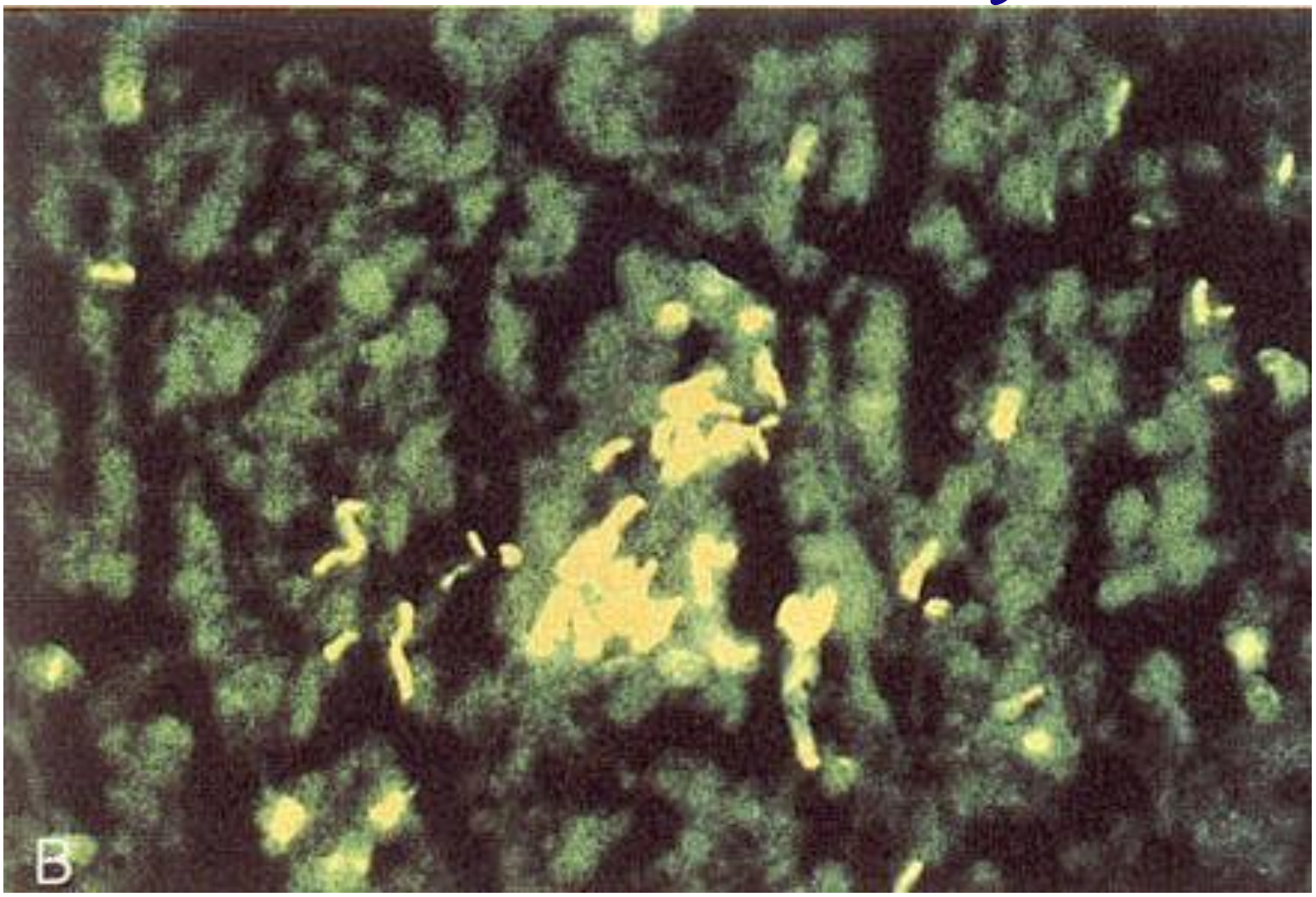
2. Molecular tests: • For rapid detection.

- M.TB. is not classified **as either Gram + or Gram-** because it does not have the chemical characteristics of either, although the bacteria do contain peptidoglycan (murein) in their cell wall. If a Gram stain is performed on M.TB., it stains very weakly Gram-positive or not at all (referred to as "ghosts").

- The bacilli will appear red in sharp contrast to the blue colour stained background .



Mycobacterium Tuberculosis Stained with Fluorescent Dye



Laboratory Diagnosis

- ***Cultivation:***

- *Media include: - Lowenstein-Jensen (L-J) medium – Middlebrook's medium*

Incubation is at 35-37 °C in 5-10% CO2 for up to 8 weeks.

- *If culture negative and acid-fast Positive , a set of inoculated media should be incubated at 24-33°C for 12 weeks.*

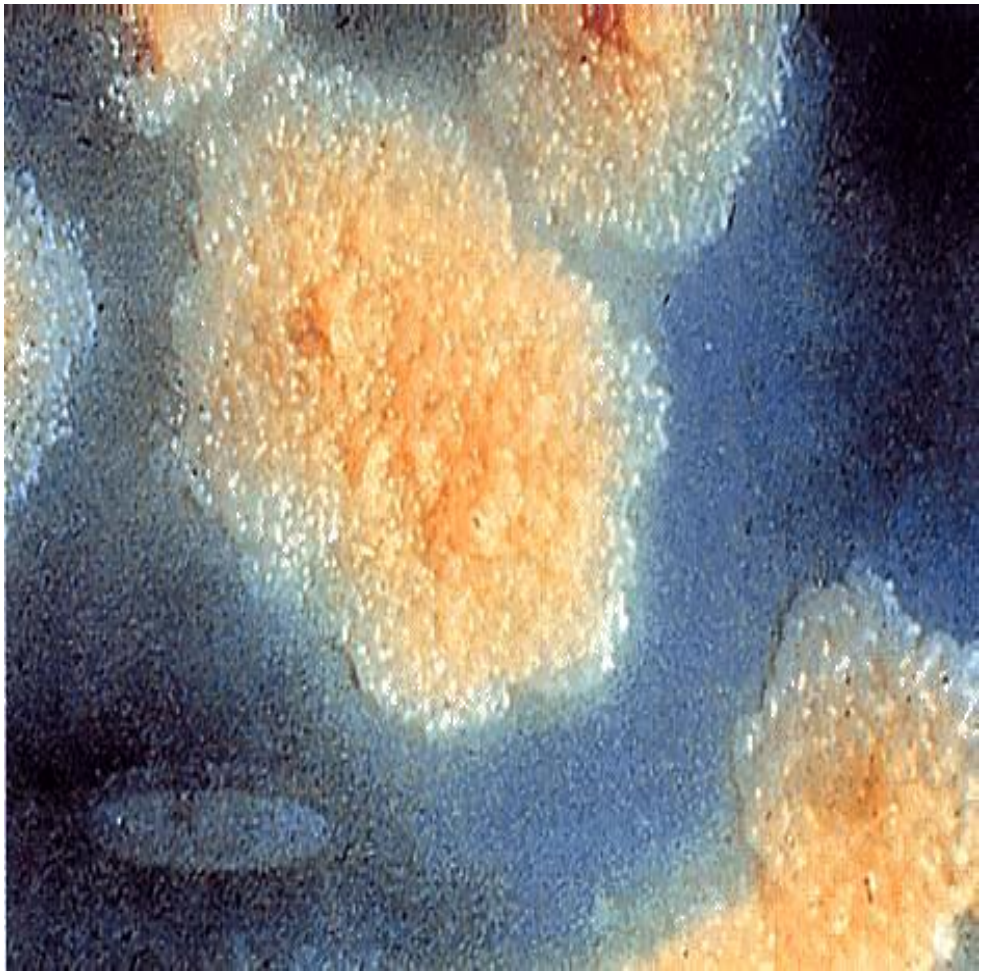
- **Medically important mycobacterial species grow slowly. Their generation (doubling) time ranges from a few hours to >2 days, compared to 40–60 minutes for most other bacteria.**

- **Colonies may take 2–3 weeks to develop from the time of inoculation of culture media.**

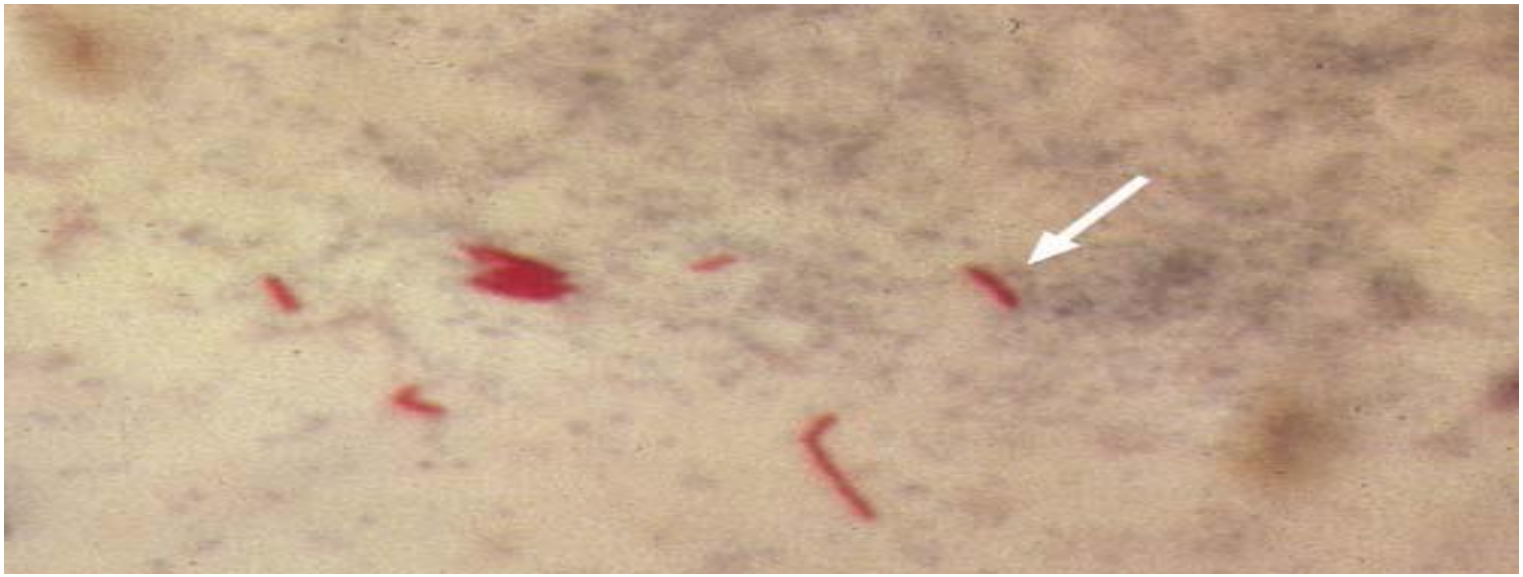
- **Mycobacteria do not grow on ordinary laboratory media but grow well on Löwenstein-Jensen (LJ) medium, containing egg, asparagine, glycerol and malachite green. The latter inhibits contaminants. Two media are used to grow M.TB. Middlebrook's medium**

	Colonial morphology on	
	Lowenstein-Jensen medium	**Stonebrink's medium**
M. tuberculosis	**Rough**=dry and irregular surface **Tough**=Hard and difficult to emulsify **Buff**=in colour (creamy to light yellow)	The same characteristics are shown on this medium

Eight Week Growth of Mycobacterium tuberculosis on Lowenstein-Jensen Agar



In order to detect Mycobacterium tuberculosis in a sputum sample, in excess of 10,000 organisms per ml of sputum are needed to visualize the bacilli with a 100X microscope objective. One acid-fast bacillus/slide is regarded as "suspicious" of an M.TB. infection.



Mycobacterium tuberculosis. **Acid-fast stain.**

Cell Wall Structure

Over 60% of the mycobacterial cell wall is lipid. The lipid fraction of M.TB's cell wall consists of three major components.

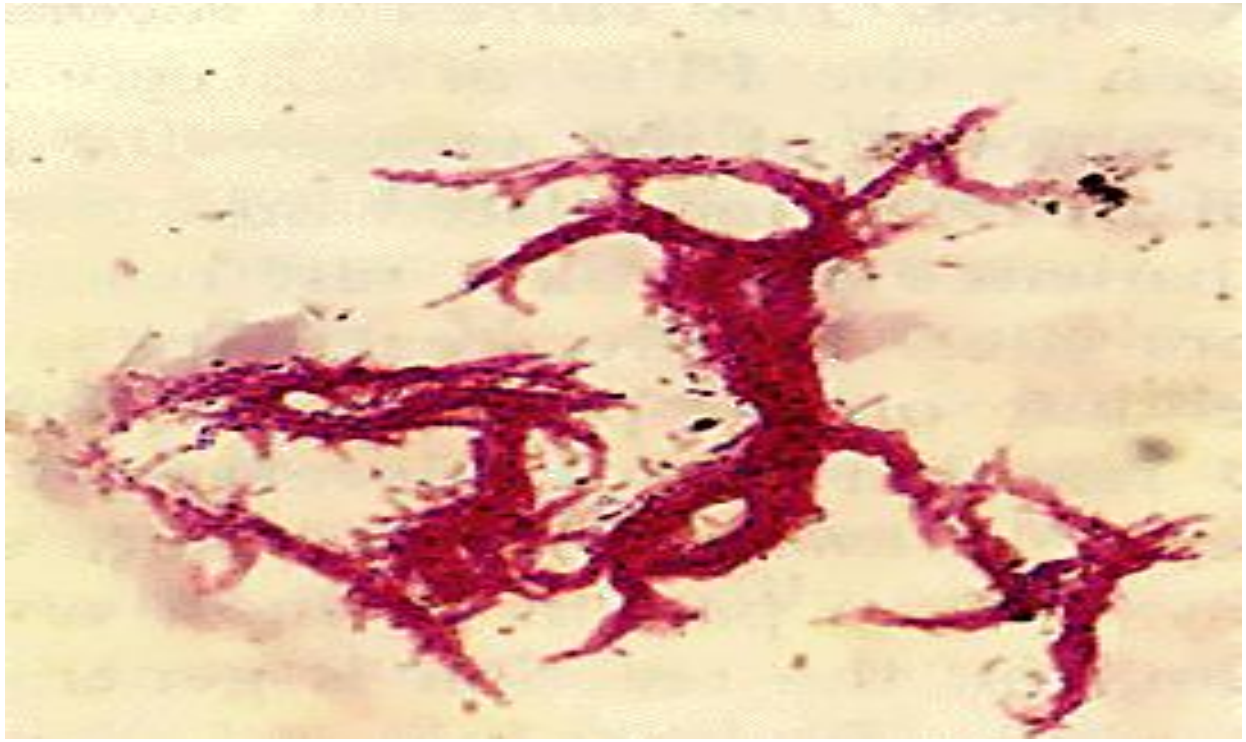
Mycolic acids are unique alpha-branched lipids found in cell walls of Mycobacterium and Corynebacterium.

Mycolic Acids are thought to be a significant determinant of virulence in M.TB. Probably, they prevent attack of the mycobacteria by cationic proteins, lysozyme and oxygen radicals in the phagocytic granule. They also protect extracellular mycobacteria from complement deposition in serum.

Cord Factor is responsible for the serpentine cording (chains of cells form distinctive serpentine cord). Cord factor is toxic to mammalian cells and is also an inhibitor of PMN migration. Cord factor is most abundantly produced in virulent strains of M.TB.

Wax-D in the cell envelope

❑When smear is prepared from **culture**, the bacilli appear short, thick and tend to arrange in a sort of bundles (a phenomenon referred to as a **cord formation** due to the cord factor.



NOTE: cord growth (serpentine arrangement) of virulent strains

In summary, the high concentration of lipids in the cell wall of Mycobacterium tuberculosis has been associated with these properties of the bacterium:

- Impermeability to stains and dyes
- Resistance to many antibiotics
- Resistance to killing by acidic and alkaline compounds
- Resistance to osmotic lysis via complement deposition
- **Resistance to lethal oxidations and survival inside of macrophages**

Predisposing factors for TB infection include:

- Close contact with large populations of people, i.e., schools, nursing homes, dormitories, prisons, etc.
- Poor nutrition
- IV drug use
- Alcoholism

HIV infection is the predisposing factor for M.TB. infection.

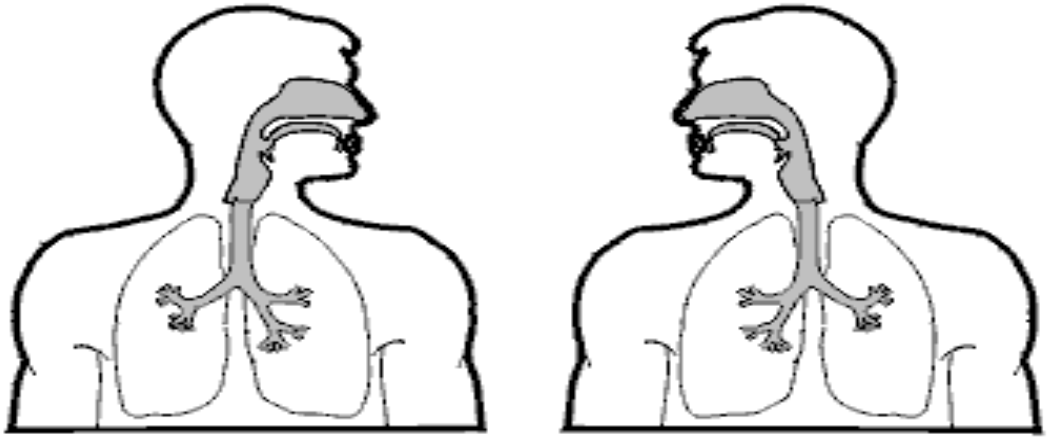
Tuberculosis: Infection vs Disease

TB disease in lungs	TB Infection
M.TB. present	M.TB. present
Tuberculin skin test positive	Tuberculin skin test positive
Chest X-ray usually reveals lesion	Chest X-ray normal
Sputum smears and cultures positive	Sputum smears and cultures negative
Symptoms such as cough, fever, weight loss	No symptoms
Often infectious before treatment	Not infectious
Defined as a case of TB	Not defined as a case of TB

Stages of the Disease:-

Stage 1

Droplet nucleiare inhaled



Spread of droplet nuclei from one individual to another. After droplet nuclei are inhaled, the bacteria are nonspecifically taken up by alveolar macrophages. However, the macrophages are not activated and are unable to destroy the intracellular organisms

Stage 2

Begins 7-21 days after initial infection. M.TB. multiplies virtually unrestricted within inactivated macrophages until the macrophages burst.

Stage 3

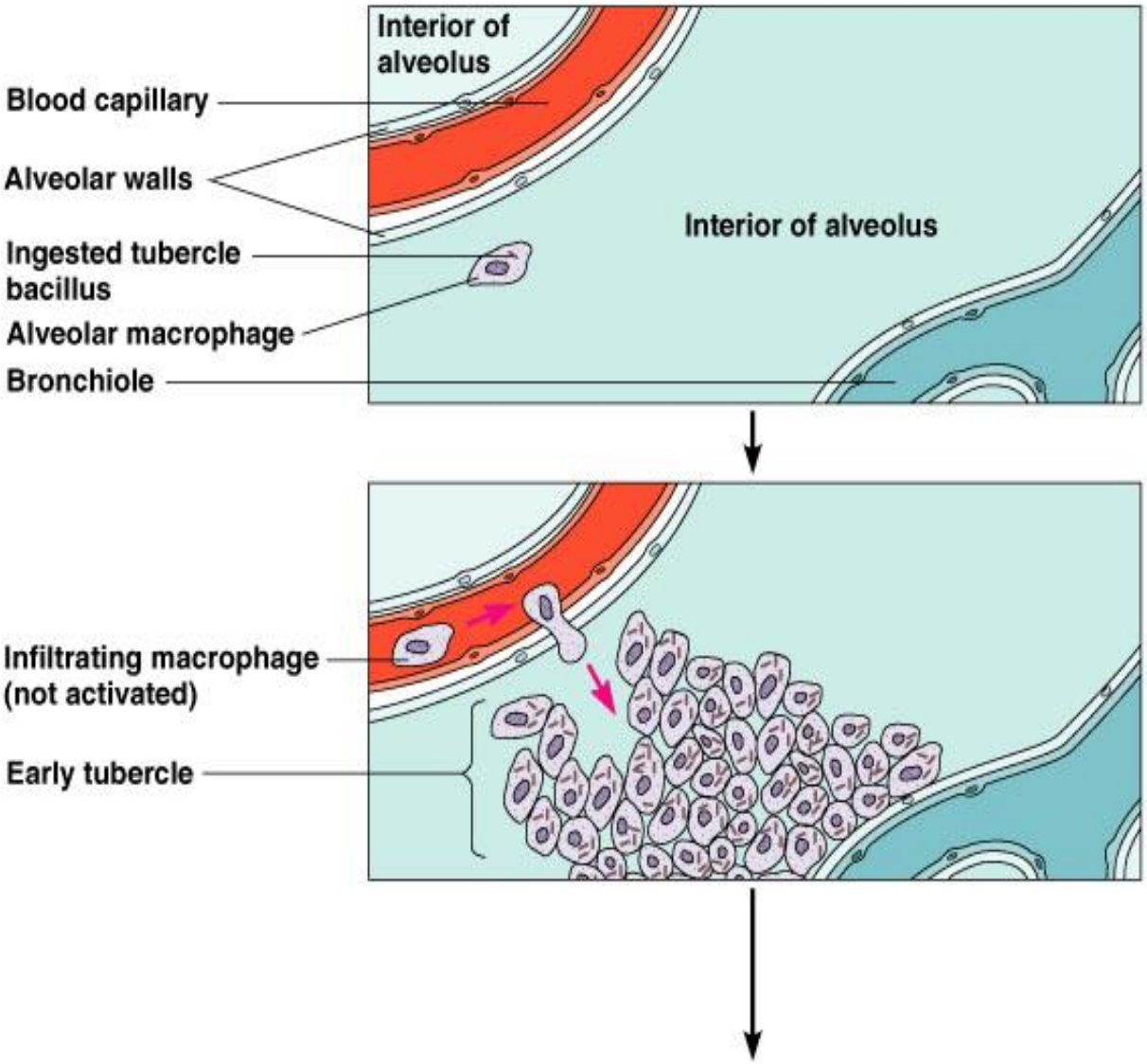
lymphocytes begin to infiltrate.

The lymphocytes, specifically T-cells, recognize processed and presented M.TB

T-cell activation and the liberation of cytokines including gamma interferon (IFN).

tubercle formation begins. The center of the tubercle is characterized by "caseation necrosis" meaning semi-solid or "cheesy" consistency

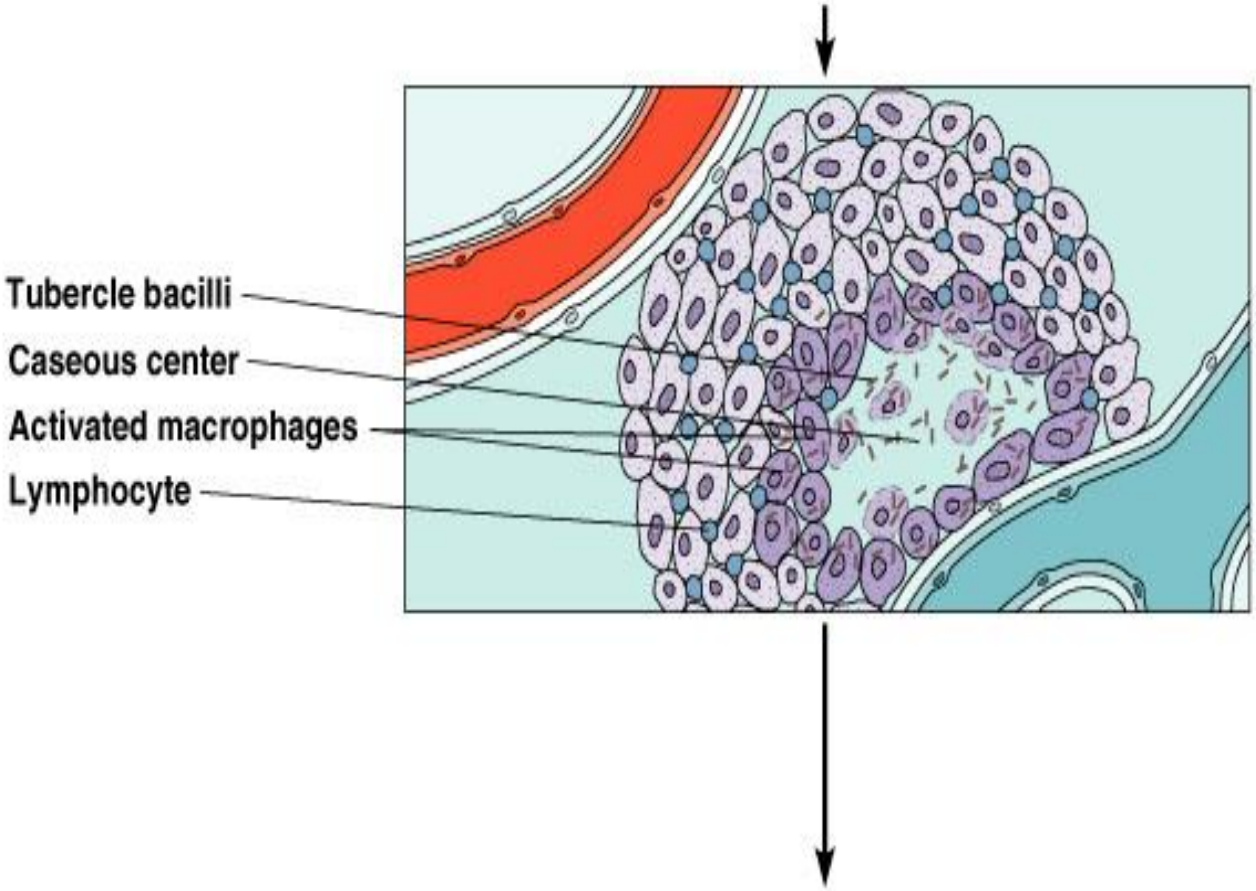
Progression of TB



1. Tubercle bacilli that reach the alveoli of the lung (Figure 24.2) are ingested by macrophages, but some often survive. Infection is present, but no symptoms of disease.

2. Tubercle bacilli multiplying in macrophages cause a chemotactic response that brings additional macrophages and other defensive cells to the area. These form a surrounding layer and, in turn, an early tubercle. Most of the surrounding macrophages are not successful in destroying bacteria but release enzymes and cytokines that cause a lung-damaging inflammation.

Progression of TB



3 After a few weeks, disease symptoms appear as many of the macrophages die, releasing tubercle bacilli and forming a *caseous center* in the tubercle. The aerobic tubercle bacilli do not grow well in this location. However, many remain dormant (latent TB) and serve as a basis for later reactivation of the disease. The disease may be arrested at this stage, and the lesions become calcified.

Stage 4

The growing tubercle may invade a bronchus. If this happens, M.TB. infection can spread to other parts of the lung.

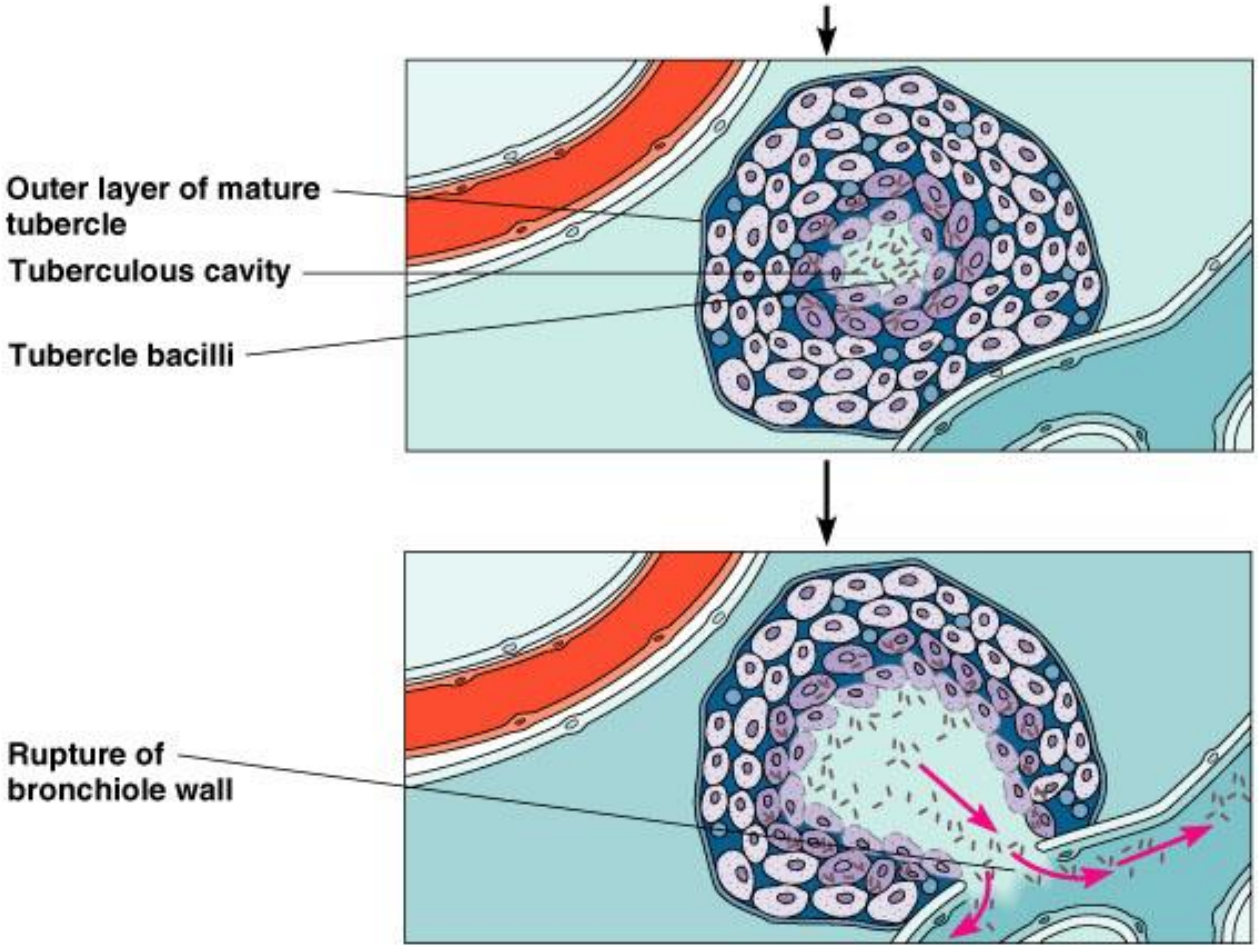
milliary tuberculosis. The name "milliary" is derived from the fact that metastasizing tubercles are about the same size as a millet seed,

Stage 5

The caseous centers of the tubercles liquify.

the walls of nearby bronchi to become necrotic and rupture.

Progression of TB



4 In some individuals, disease symptoms appear, as a mature tubercle is formed. The disease progresses as the caseous center enlarges in the process termed *liquefaction*. The caseous center now enlarges and forms an air-filled *tuberculous cavity* in which the aerobic bacilli multiply outside macrophages.

5 Liquefaction continues until the tubercle ruptures, allowing bacilli to spill into a bronchiole (see Figure 24.2) and thus be disseminated throughout the lungs and then to the circulatory and lymphatic systems.

Symptoms include : fever

coughing (often with blood)

weight loss

malaise (loss of energy)

→ progressive lung damage

Systemic TB

Can infect any area of the body including:

- **Bones and joints**
- **Internal organs**
- **Brain**

Progression of TB

Bacteria in tubercles may survive for decades (latency)

Suppression of immune system may allow bacteria to break out of lesions and multiply (reactivation)

Old age, cancer, immunosuppressive drugs and HIV infection can lead to reactivation

Diagnosis of TB

typical chest X-ray

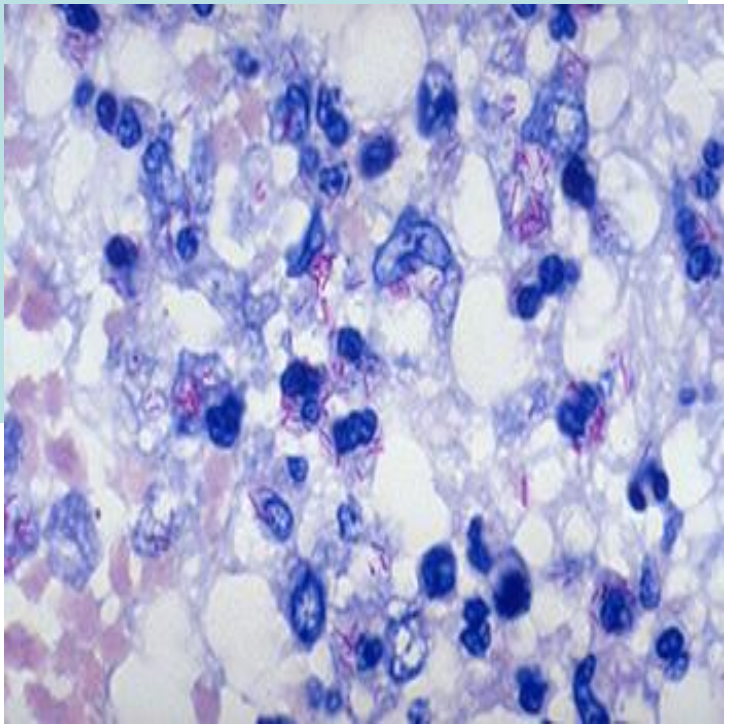
#Acid-fast staining of sputum samples via the Ziehl-Neelsen method

fluorescent microscope is a sensitive but not very specific method of detection

#cultured on LJ slopes

#molecular techniques (e.g. PCR)

#Biopsies

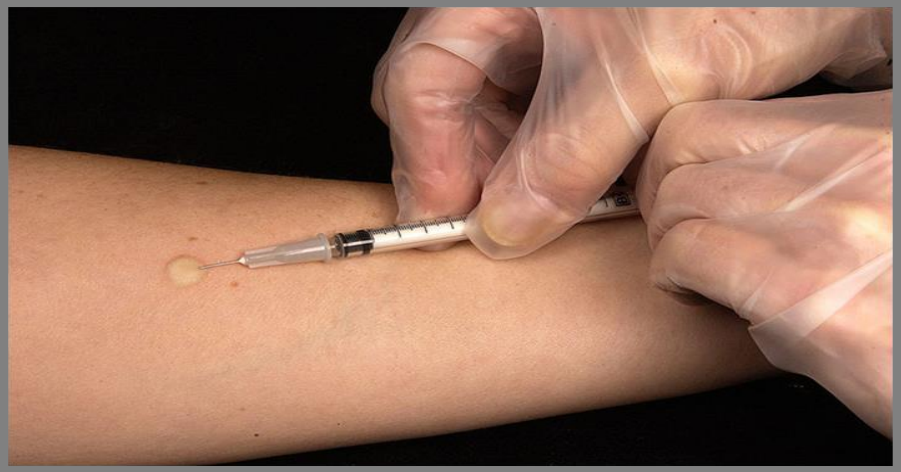


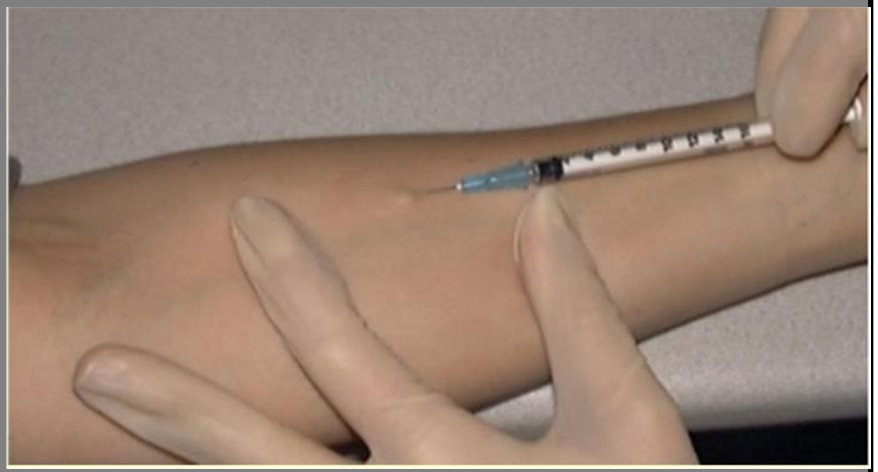
The Mantoux test

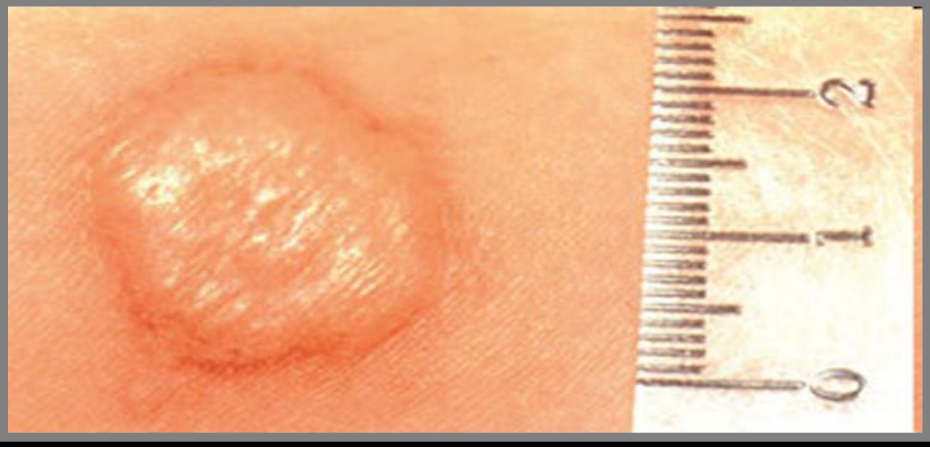
- *also known as the Tuberculin Sensitivity Test, is a diagnostic tool for tuberculosis.*
- *Tuberculin is a glycerol extract of the tubercle bacillus.*
- *A standard dose of 5 Tuberculin units (0.1mL) is injected intradermally and read 48 to 72 hours later.*
- *The reaction is read by measuring the diameter of induration across the forearm in millimeters.*

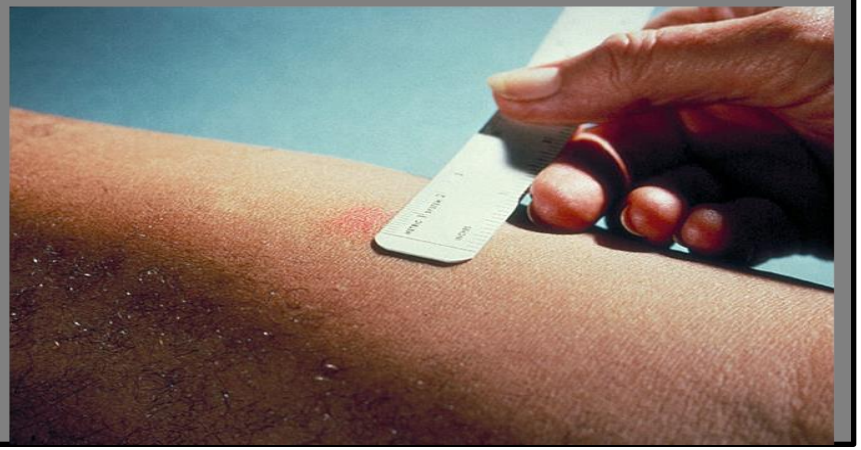
The Mantoux test

- *It is considered positive if the induration 10mm or more in daimeter.*









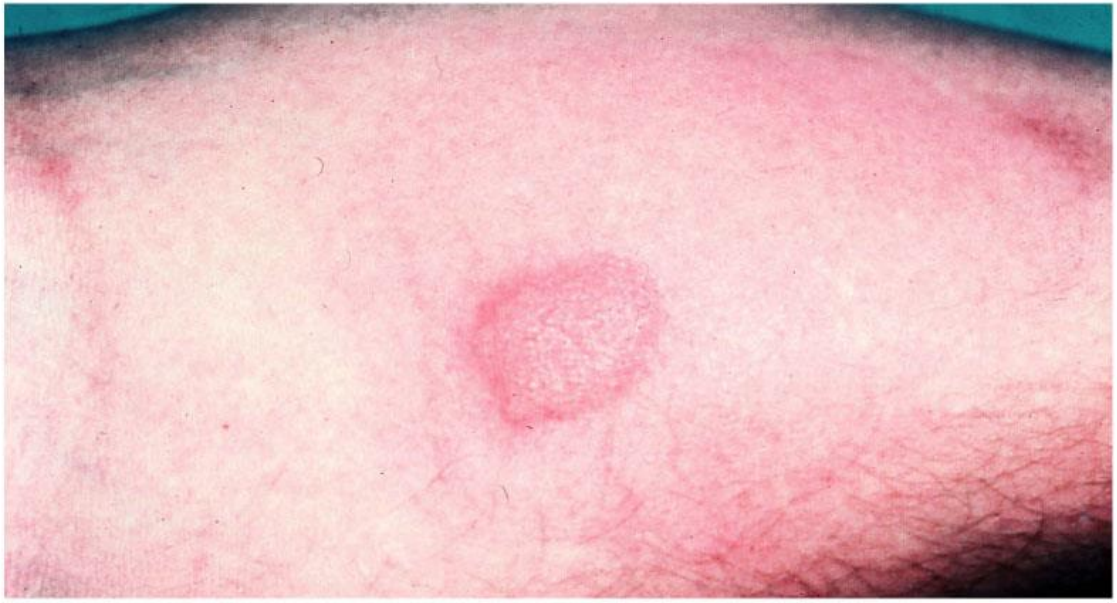
The Mantoux test

- *Apositive test indicates that an individual has been infected in the past.*
- *It does not imply that active disease or immunity to disease is present.*
- *Tuberculin positive person are of risk of developing disease from reactivation of the primary infection.*

Diagnosis of TB

Skin Testing is performed as the tuberulin or Mantoux test. skin test - injection of *M. tuberculosis* proteins **(tuberculin)**

- **positive test** leads to red area at injection site



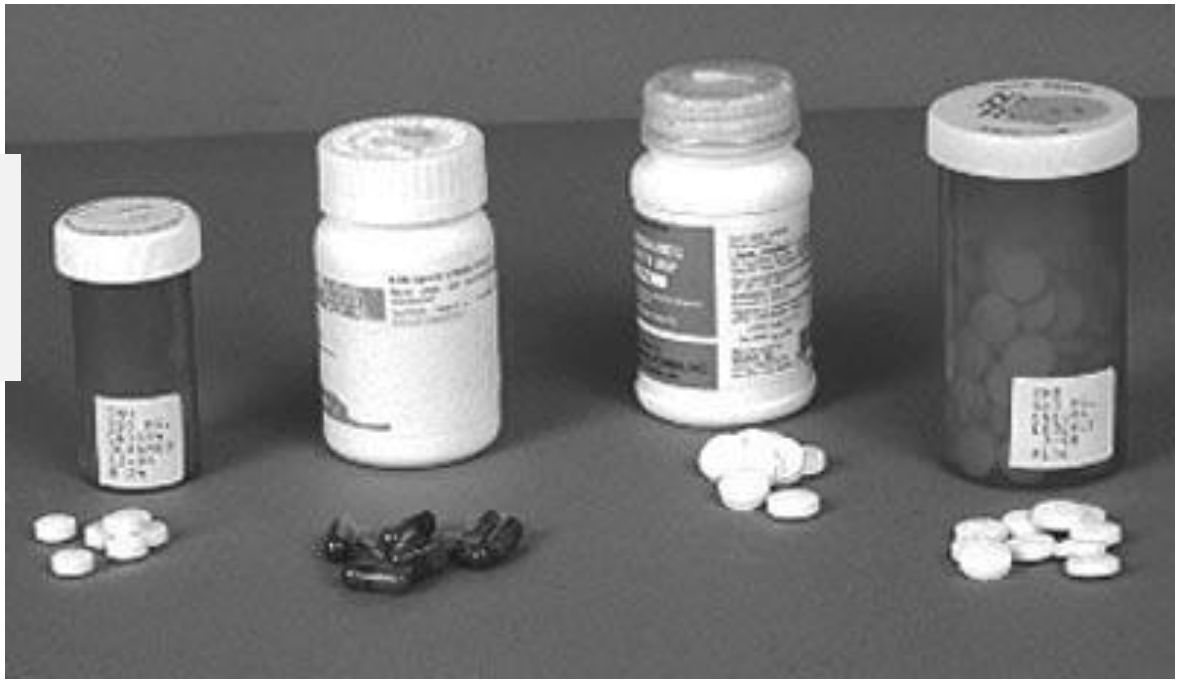
PPD = Purified Protein Derivative from *M. tuberculosis*

BCG (bacille Calmette-Guerin) = attenuated *M. bovis*

Treatment of TB

Treatment requires the use of multiple drugs for long periods of time (6–9 months). Commonly used regimens include 2 months of isoniazid, rifampicin, pyrazinamide and ethambutol followed by 4–6 months of the first two drugs alone.

Multi-drug resistant M. tuberculosis is a major public health problem worldwide.



Drugs used to treat TB disease. From left to right isoniazid, rifampin, pyrazinamide, and ethambutol

Prevention

A vaccine against M.TB. is available.

It is called BCG (Bacillus of Calmette and Guerin).

BCG consists of a live attenuated strain derived from Mycobacterium bovis.

The vaccine is not 100% effective.

Mycobacterium avium

Also known as M avium intracellulare MAI.

These organism grow optimally at 41^{0}C and produce smooth, soft, nonpigmented colonies.

Cultured from water, soil, food, and animals, including birds.

Cause disease in immunocomptent human.

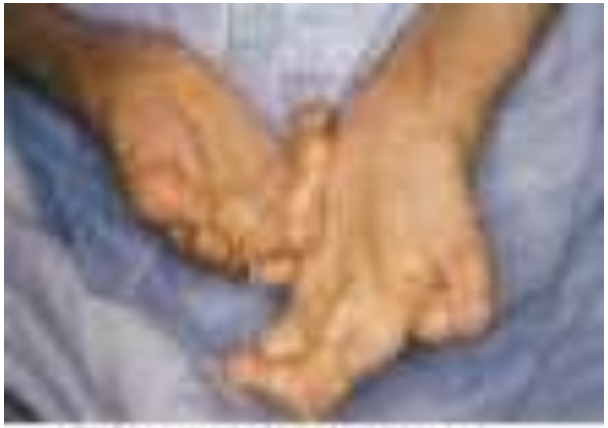
Mycobacterium leprae

- *Typical acid-fast bacilli, singly, in parallel bundles, or in globular masses,*
- *It cause leprosy.*
- *Daignosis: by scraping from skin or nasal mucosa are smeared a slide and stained by the Ziel-Neelsen technique.*
- *Biopsy of skin for histological study.*

Mycobacterium leprae

- Causes leprosy or Hansen's Disease
- Infection of the skin, mucous membranes and peripheral nerves
- Most cases are from warm climates
- Bacteria infect the cooler areas of the body (ears, nose, eyebrows, fingers, toes)
- Diagnosis made from finding acid-fast bacilli in scrapings from lesions
- Not culturable, except in mouse foot pads

Mycobacterium leprae



Disfigurement of hands in leprosy.
M.G.Lebwohl, Atlas of the Skin
and Systemic Diseases, 1995

Mycobacterium leprae Infections

Physiology and Structure

Weakly gram-positive, strongly acid-fast bacilli.

Lipid-rich cell wall.

Unable to be cultured on artificial media.

Diagnosis made with specific skin test (tuberculoid form of disease) or acid-fast stain (lepromatous form).

Virulence

Capable of intracellular growth.

Disease primarily from host response to infection.

Epidemiology

Rare in United States but common in other countries (e.g., Asia, Africa).

Armadillos are naturally infected and represent an indigenous reservoir.

Lepromatous form of disease, but not the tuberculoid form, is highly infectious.

Person-to-person spread by direct contact or inhalation of infectious aerosols.

People in close contact with patients who have lepromatous disease are at greatest risk.

Mycobacterium leprae Infections (cont.)

Diseases

Tuberculoid form of leprosy.

Lepromatous form of leprosy.

Intermediate forms of leprosy.

Diagnosis

Microscopy is sensitive for the lepromatous form but not the tuberculoid form.

Skin testing required to confirm tuberculoid leprosy. Culture cannot be used.

Treatment, Prevention, and Control

Dapsone with or without rifampin is used to treat the tuberculoid form of disease; clofazimine is added for the treatment of the lepromatous form. Therapy is prolonged.

Dapsone is recommended for long-term prophylaxis in treated patients.

Disease is controlled through the prompt recognition and treatment of infected people.

Thanks for your Attention!

Lec:9

Chlamydia

***Structure and Chemical Composition**

*** Growth and Metabolism**

***What is chlamydia?**

***Developmental Cycle**

***Symptoms and signs**

***Diagnosis of chlamydia**

Treatment

Chlamydiae that infect humans are divided into three species, *Chlamydia trachomatis, Chlamydia (Chlamydophila) pneumoniae,* and *Chlamydia (Chlamydophila) psittaci*, on the basis of antigenic composition, intracellular inclusions, sulfonamide susceptibility, and disease production. Other chlamydiae infect animals **but rarely if ever infect humans**. All chlamydiae exhibit similar morphologic features and multiply in the cytoplasm of their host cells by a distinctive developmental cycle. The chlamydiae can be viewed as gram-negative bacteria that lack mechanisms for the production of metabolic energy and cannot synthesize adenosine triphosphate **(ATP).** This restricts them to an intracellular existence, where the host cell furnishes energy-rich intermediates. Thus, chlamydiae are **obligate intracellular parasites**.

Structure and Chemical Composition

In chlamydiae, the outer cell wall resembles the cell wall of gram-negative bacteria. It has a relatively high lipid content. It is rigid but does not contain a typical bacterial peptidoglycan. Penicillin-binding proteins occur in

chlamydiae, and chlamydial cell wall formation is inhibited by penicillins and other drugs that inhibit transpeptidation of bacterial peptidoglycan. Lysozyme has no effect on chlamydial cell walls. Both DNA and RNA are present in elementary and RBs. The RBs contain about four times as much RNA as DNA, whereas the EBs contain about equal amounts of RNA and DNA.

Growth and Metabolism

Chlamydiae require an intracellular habitat because they are unable to synthesize ATP and depend on the host cell for energy requirements. Chlamydiae grow in cultures of a variety of eukaryotic cells lines. All types of chlamydiae proliferate in embryonated eggs, particularly in the yolk sac. The replication of chlamydiae can be inhibited by many antibacterial drugs.

Cell wall inhibitors such as penicillins and cephalosporins result in the production of morphologically defective forms but are not effective in clinical diseases.

Inhibitors of protein synthesis (tetracyclines, erythromycins) are effective in most clinical infections. *C trachomatis* strains are susceptible to inhibition by sulfonamides. Aminoglycosides are noninhibitory

What is chlamydia?

Chlamydia is a sexually transmitted infection (STI) caused by the bacterium *Chlamydia trachomatis*. The bacterium infects the “wet” linings (mucous membranes) of the body. Chlamydia can infect the genital tracts, including the cervix, uterus, fallopian tubes, urethra (the tube that allows urine and semen to pass out of the body) and epididymis (a tube in the testicle that stores and carries sperm).

It can also infect the throat (pharynx), anus and rectum. In addition, it can infect the eyes through contact with infected discharge. Chlamydia is a common

STD that can infect both men and women. It can cause serious, permanent damage to a woman's reproductive system. This can make it difficult or impossible for her to get pregnant later on.

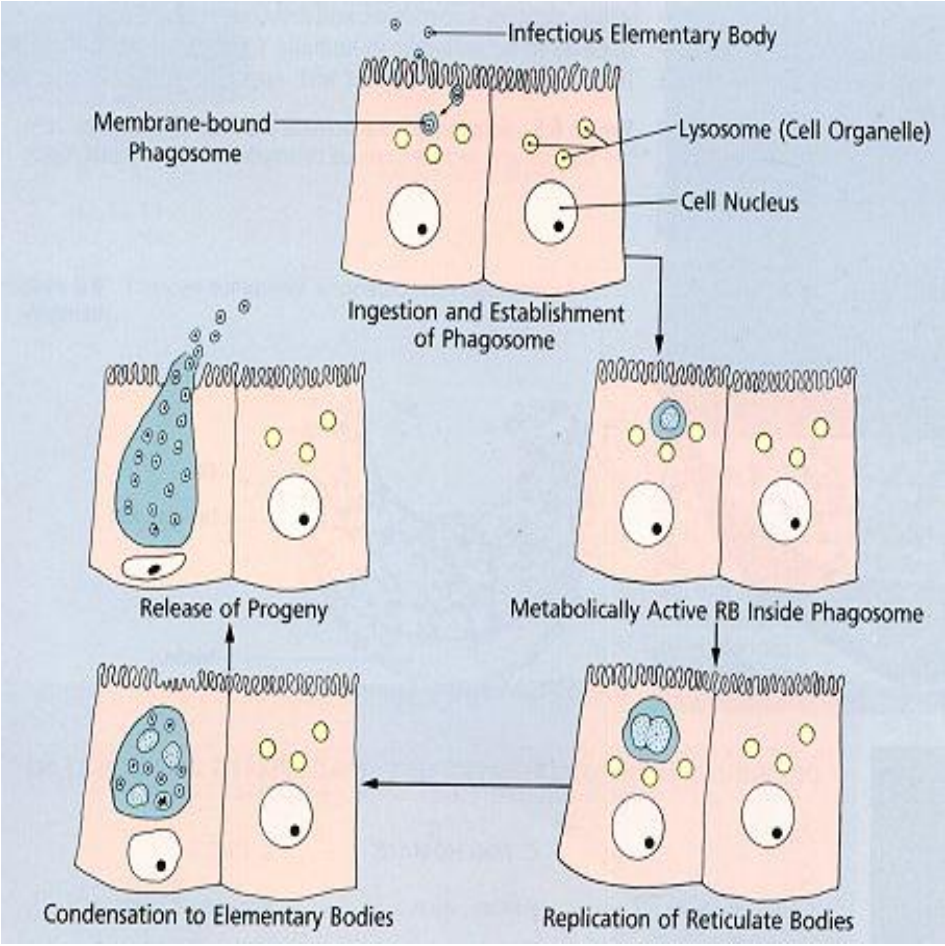
Chlamydia can also cause a potentially fatal ectopic pregnancy (pregnancy that occurs outside the womb). STI caused by bacterium *Chlamydia trachomatis* primarily targets cells of mucous membranes including urethra, vagina, cervix and endometrium (mouth and throat) one of most commonly reported bacterial STDs

Developmental Cycle

All chlamydiae share a common and unique biphasic developmental cycle. The environmentally stable infectious particle is a small cell called the elementary body (EB). These are about 0.3 µm in diameter with an electrondense nucleoid. The EB membrane proteins have highly cross-linked membrane proteins. The EBs have a high affinity for host epithelial cells and rapidly enter them. There appear to be multiple adhesins, receptors, and mechanisms of entry. Heparan sulfate–like proteoglycans on the surface of *C trachomatis* are likely possibilities for mediating at least the initial interaction between EBs and host cells. Other potential adhesins include the major outer membrane protein (MOMP), glycosylated MOMP, and other surface proteins.

The mechanisms thought to mediate entry into the host cell also varied. EBs are usually seen attached near the base of microvilli, where they are subsequently engulfed by the host cell. More than one mechanism appears to be functional: receptor-mediated endocytosis into clathrin-coated pits and pinocytosis via noncoated pits. Lysosomal fusion is inhibited, creating a protected membrane-bound environment around the chlamydiae. Shortly after entry into the host cell, the disulfide bonds of the EB membrane proteins are no

longer cross-linked, and the EB is reorganized into a larger structure called a reticulate body (RB) measuring about 0.5–1 μm and devoid of an electron-dense nucleoid. Within the membrane-bound vacuole, the RB grows in size and divides repeatedly by binary fission. Eventually, the entire vacuole becomes filled with EBs derived from the RBs to form a cytoplasmic inclusion. The newly formed EBs may be liberated from the host cell to infect new cells. The developmental cycle takes 24–48 hours.



Symptoms and signs: Most people do not have any symptoms and are unaware that they have the infection. Approximately 70% of women and 50% of men with the infection do not have any symptoms. Appear between 1 and 3

weeks after exposure (may not emerge until much later),"silent disease", 70-75% asymptomatic women.

In women, chlamydia can infect the cervix or urethra. Symptoms can include:

• pain when urinating

• discharge from the vagina

• pain in the lower abdomen

• pain or bleeding during or after sex

• bleeding between periods

In men, chlamydia can infect the urethra,Symptoms can include:

• pain when urinating

• discharge from the penis

In both sexes, chlamydia can infect the anus (there are usually no symptoms). For women and men, if untreated Chlamydia can affect your ability to have children, and may cause ongoing pelvic pain.

Ecology: Chlamydia form two main ecological groups.

Infect only humans :Subgroup A(trachoma, inclusion conjunctivitis, and lymphogranuloma venereum).

Zoonotic Infections: Subgroup B(Respiratory tract infections)

Classification

Chlamydiae are classified according to their pathogenic potential, host range, antigenic differences, and other methods. Three species that infect humans: ***C. trachomatis, C. psittaci, C. pneumoniae***

Chlamydia pneumonia

This bacterium was first recognized in 1983 as a respiratory pathogen, after isolation from a college student with pharyngitis. Pneumonia or bronchitis, gradual onset of cough with little or no fever. Less common presentations are pharyngitis, laryngitis, and sinusitis. Most recognized species of Chlamydia important respiratory pathogen (acute respiratory disease, pneumonia, and pharyngitis), Implicated in asthma.

Chlamydia trachomatis

- Most commonly sexually transmitted bacterial pathogen in U.S.

  Only HPV is a more commonly sexually transmitted disease

- Adult males:(Non-gonococcal urethritis (NGU), Epididymitis and prostatitis).
- Adult females:(Urethritis, follicular cervicitis, endometritis, proctitis, salpingitis, PID and perihepatitis (Fitz-Hugh-Curtis syndrome)
  - Major cause of sterility in U.S.
  - May be transmitted to newborns during delivery
- Other sites of infection
- Trachoma – infection of the conjunctiva, resulting in scarring and blindness (Mostly in India and Egypt)
- Lymphogranuloma venereum – STD found in immigrants
- **Laboratory Diagnosis**

- Direct microscopic examination to find EBs .
- Cell culture
- Enzyme immunoassay
- Nucleic acid probes with and without amplification (PCR)
- Serologic (antibody) assay.

Diagnosis of chlamydia :: - Urine sample,-Swab taken from vagina and -Swab taken from opening of the urethra at the tip of the penis

Chlamydia psittaci (Causes psittacosis (parrot fever)**,** Identification based on history of close contact with birds and serologic evaluation.Person-to-person transmission by respiratory secretions.

Treatment: It is essential that chlamydial infections be treated simultaneously in both sex partners and in offspring to prevent reinfection. Tetracyclines (eg, doxycycline) are commonly used in nongonococcal urethritis and in nonpregnant infected women. Azithromycin is effective and can be given to pregnant women. Topical tetracycline or erythromycin is used for neonatal ***N gonorrhoeae*** infections but may not effectively prevent neonatal C trachomatis infection. Systemic therapy should be used for inclusion conjunctivitis because topical therapy may not cure the eye infections or prevent respiratory disease.

-Repeat infection with chlamydia is common. You should be tested againa bout three months after you are treated, even if your sex partner(s) was treated.

What happens if I don't get treated?

If you are a woman, untreated chlamydia can spread to your uterus and fallopian tubes (tubes that carry fertilized eggs from the ovaries to the uterus).

This can cause pelvic inflammatory disease (PID). PID often has no symptoms, however some women may have abdominal and pelvic pain. Even if it doesn't cause symptoms initially, PID can cause permanent damage to your reproductive system.

PID can lead to long-term pelvic pain, inability to get pregnant, and potentially deadly ectopic pregnancy (pregnancy outside the uterus).