AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

FACULTY: General Medicine
SUBJECT: Medical microbiology - 2

Lecture 4.

The pathogenic bacteria including genus of *Corynebacterium, Bordetella, Haemophilus, Gardnerella, Legionella, Mycobacterium, Actinomyces* and *Nocardia*

Lecture plan:

1. Bacteria from the genus *Corynebacterium*. Morpho-biological characteristics of diphtheria causative agent. Pathogenicity factors. Mechanism of action of C.diphtheriae toxin. Pathogenesis of diphtheria. Microbiological diagnosis of diphtheria. Specific principles of prevention and treatment.

2. *Bordetellas*, classification, morpho-biological characteristics. Pertussis agents, their pathogenicity factors. Disease pathogenesis, microbiological diagnosis, specific prevention and treatment principles.

3. Hemophilic bacteria. *H.influenzae*, morpho-biological characteristics, pathogenicity factors. Role in human pathology. *H.ducreyi*, morpho-biological characteristics and microbiological diagnosis.

4. *Legionella*, morpho-biological characteristics, pathogenicity factors. Legionellosis pathogenesis, clinical forms, microbiological diagnosis.

5. *Gardnerella vaginalis*, morpho-biological characteristics, pathogenetic characteristics, microbiological diagnosis.

6. General characteristics, classification of bacteria from the genus *Mycobacterium*.

- Tuberculosis agents, morpho-biological characteristics, pathogenicity factors. Drug resistance. Multidrug-resistant (MDR), extensively drug-resistant (XDR), pandrug-resistant (PDR). Pathogenesis of the disease. Microbiological diagnostics. Specific prevention and treatment of tuberculosis. BCG vaccine.

- The causative agent of leprosy. Morpho-biological characteristics. Clinical forms of leprosy. Microbiological diagnostics.

7. *Actinomycetes*, classification, morpho-biological characteristics, pathogenicity factors. Pathogenesis, clinical forms and microbiological diagnosis of actinomycosis.

8. *Nocardia*, their role in human pathology.

Corynebacterium - Taxonomy

(Domain): Bacteria

(Kingdom): Actinomycetota

(Class): Actinomycetia

(Order): Mycobacteriales

(Family): Corynebacteriaceae

(Genus): Corynebacterium

: (Species): ***C. diphtheriae***

MORPHOLOGY

Special stains for demonstrating the granules :

- Albert's stain
- Neisser's stain
- Ponder's stain

The bacilli are arranged in pairs, palisades or small groups; the bacilli lie at various angles to each other, resembling the letters, V or L;

This is called, "Chinese letter pattern" or "cuneiform pattern";

- Slender Gram-positive rods, pleomorphic; easily decolousised;
- 0.6-0.8µ diameter and 3-6 µ length;
- Irregular swelling at one or both ends ('club shaped');
- Non-capsulate, Non-sporing and nonmotile
- Granules containing polymetaphosphate are seen in the cells;
- Take up bluish purple color against lightly stained cytoplasm, when stained with Loeffler's Methylene Blue, and hence called 'Metachromatic granules';
- Also called, 'volutin granules' or 'Babes Ernst granules';
- They are often situated at poles- 'polar bodies'

CULTURAL CHARACTERISTICS

- Aerobe and facultative anaerobe;
- Optimum temperature is 37^0C
- Growth scanty on ordinary media;
- **Enrichment with**: blood, serum or egg is necessary for good growth;
- Potassium tellurite(0.04%) acts as a *'selective agent',* as it inhibits growth of most oral commensals and retards the growth of *Candida albicans* and *S.aureus;*

MEDIA FOR CULTIVATION

Blood agar

Loeffler's serum slope

Tellurite blood agar

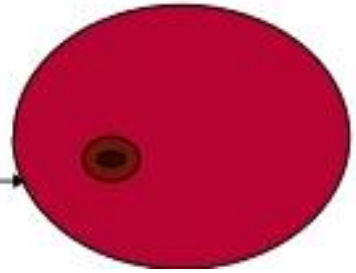
Hoyle's tellurite lysed-blood agar

COLONY CHARACTERISTICS

Tellurite blood agar:

- Growth slow; colonies seen after 48 hrs;
- The colonies are brown to black with a brown-black halo because the tellurite is reduced to metallic tellurium;
- Staphylococcus also produce such colonies

A diagrammatic representation →

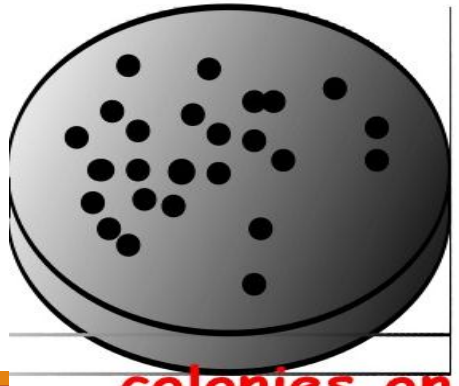


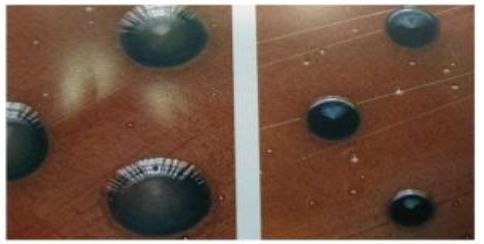
Blood agar : small, granular and gray with irregular edges; Hemolysis may or may not present;

Loeffler's serum slope:

- Very rapid growth;
- Colonies in 6-8 hrs
- Initially circular white opaque colonies and acquire yellowish tint on incubation

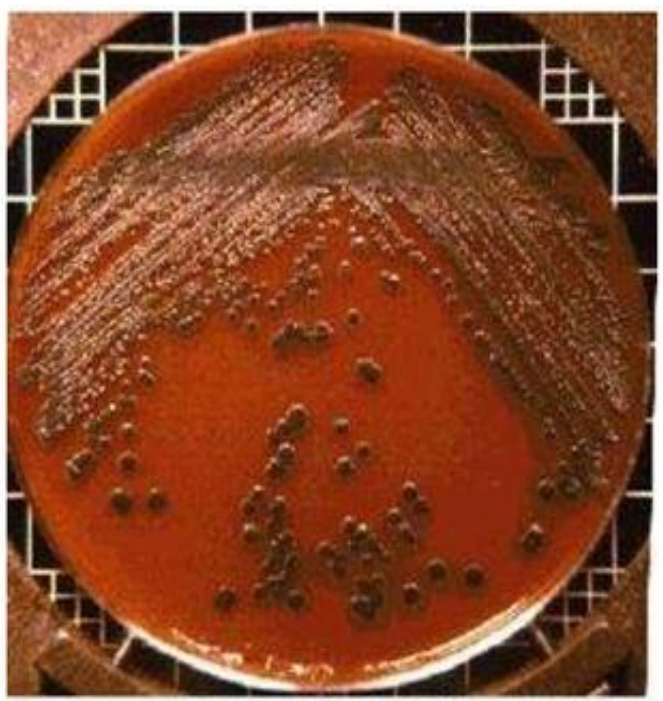
Colonial morphology

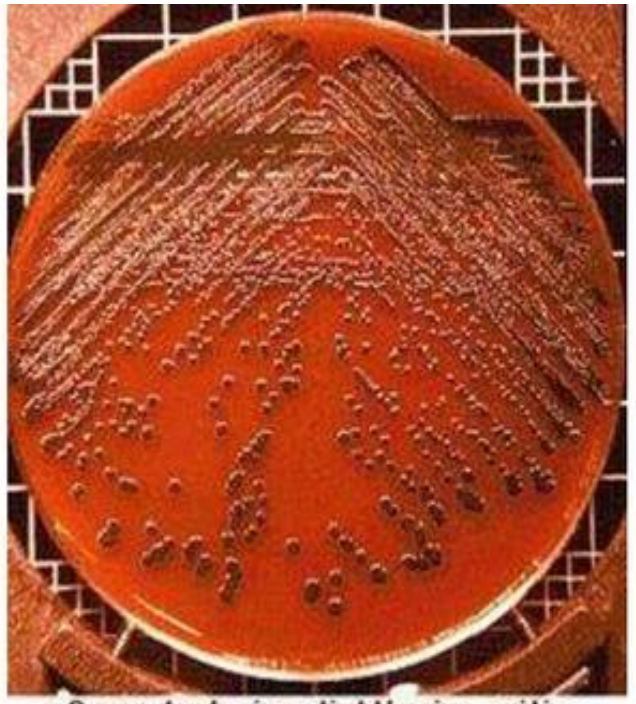




colonies on tellurite agar

C.diptheriae - biotypes cultural characteristics


Corynebacterium diphtheriae, gravis
Chocolate tellurite agar


Corynebacterium diphtheriae, mitis
Chocolate tellurite agar


Corynebacterium diphtheriae, intermedius
Chocolate tellurite agar

a) gravis **b) mitis** **c) intermedius**

BIOTYPES

Feature	gravis	intermedius	mitis
Morphology	shot rods, few granules some degree of pleomorphism	long barred forms poor granulation Pleomorphism	long curved prominent granules Pleomorphism
Colony on tellurite blood agar (48 hrs)	Daisy head colony (flat colony with raised dark centre and crenated edge; radial striations)	Frog's egg colony (dull granular centre with glistening periphery and lighter ring near edge)	Poached egg colony (shiny , flat with central elevation)
Consistency of the colonies	Brittle not easily emulsifiable	intermediate	soft, buttery easily emulsifiable
Hemolysis	**Variable**	**nonhemolytic**	**hemolytic**
Glycogen/ starch fermentation	Positive	Negative	Negative

- McLeod and Anderson classified diphtheria bacilli, based on the colony characteristics on Tellurite medium and other properties like biochemical reactions and severity of disease;
- 3 biotypes :
  - *gravis*
  - *intermedius*
  - *mitis*
- 4th biotype : *belfanti* has also been described

Corynebacterium spp. - biochemical characteristics

Species	Fermentation				Nitrate reductase
	Cystine	Carbamide	Glucose	Starch	
C.diphtheriae					
gravis	+	-	+	+	+
mitis	+	-	+	-	+
intermedius	+	-	+	-	+
belfanti	+	-	+	-	-
C.pseudodiphthericum	-	+	-	-	+
C.xerosis	-	+	+	-	+
C.ulcerans	-	+	+	-	+
C.jeikeum	-	+	+	-	-
C.sistidis	-	+	+	+	-
C.minitissumum	-	+	+	-	-

ANTIGENIC STRUCTURE AND TYPING

- Serotyping : Antigenically heterogenous
  - gravis: 13 types
  - intermedius : 4 types
  - mitis : 40 types
- Bacteriophage typing : 15 types
- Bacteriocin typing : diphtheriocin typing

Virulence Factors

1. Diphtheria toxin !!!

- blocks protein synthesis

2. Dermonecrotic toxin

- sphingomyelinase
- increases vascular permeability

3. Hemolysin

4. Cord factor -Toxic trehalose

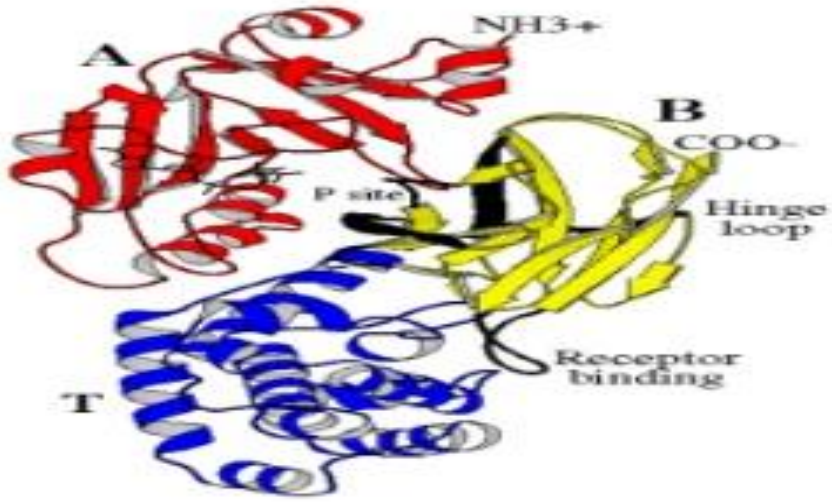
- corynemycolic acid, corynemyolenic acid

VIRULENCE FACTORS

- Virulent strains of diphtheria bacilli produce a very powerful exotoxin.
- The 'virulence' of diphtheria bacilli is due to their capacity to-
  - Establish infection and growing rapidly
  - Quickly elaborate an exotoxin
- Avirulent strains are common among convalescents , contacts and carriers, particularly those with extra-faucial infection

DIPHTHERIA TOXIN

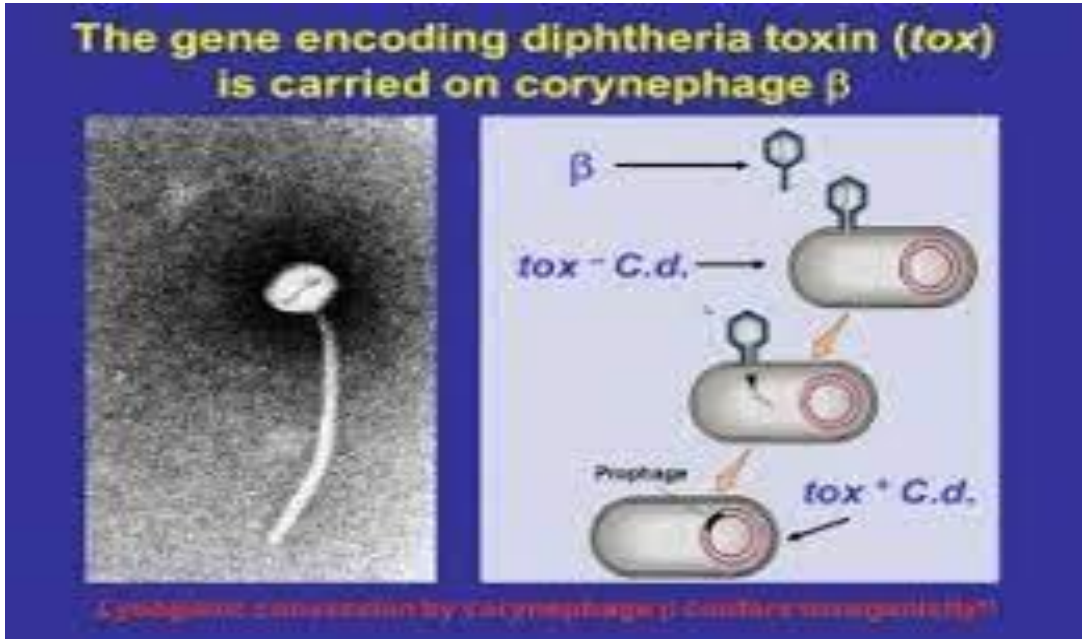
- The pathognomonic effects are due to the toxin;
- Almost all the gravis and intermedius strains and 80-85% of mitis strains are toxigenic
- Toxin is a protein;
- Mol. Wt.: 62,000
- Two fragments, A and B;
- Extremely potent :
  - 0.1 µg lethal to guinea pig
- Inactive when released

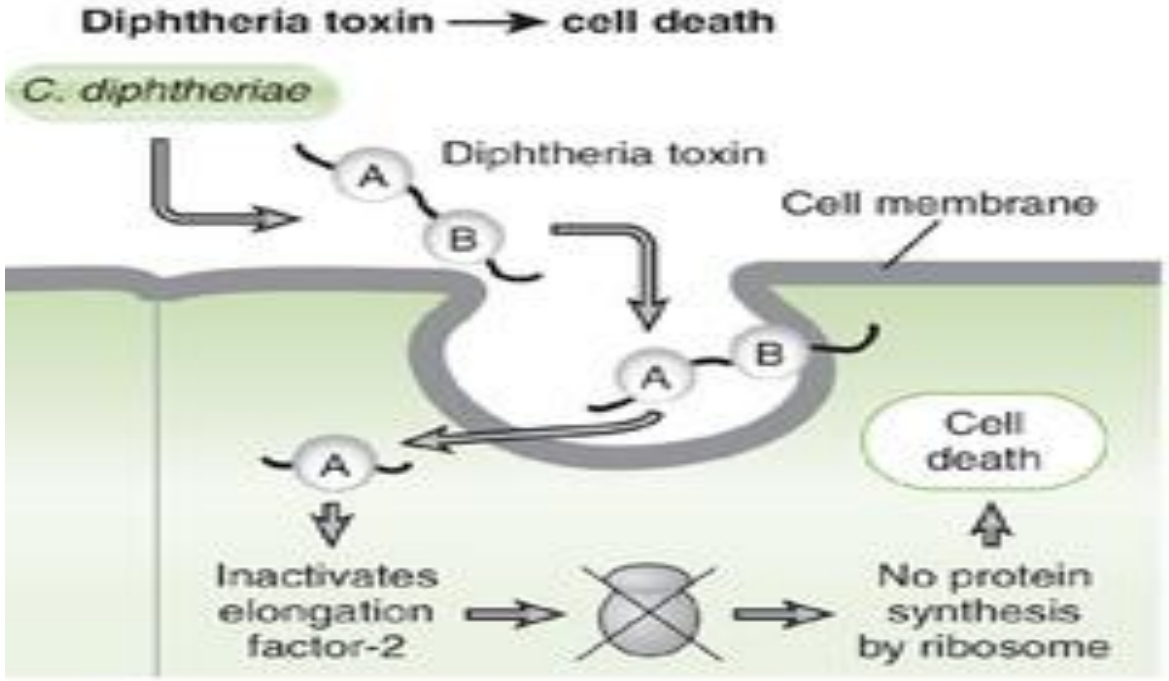


Toxin – mechanism of action

- Fragment B : binds to a cell surface receptor and helps in transport of toxin into the cell;
- After entering the cell, A subunit is released ;
- A subunit catalyses the transfer of 'adenosine diphosphate ribose (ADPR)' from NAD+
- ADPR binds with the elongation factor EF 2
- "ADPR-EF2" complex is inactive → protein synthesis stops abruptly → necrotising and neurotoxic effects of the toxin;

- **Blocks protein synthesis**
- **Protein 63Kd**
- **controlled by Tox gene**
- lysogenic phage Beta-corynephage
- **Expressed if [iron] low**
- **2 components A-B**





Toxin-systemic absorption

- The bacilli continue to produce the toxin;
- The toxin is absorbed systemically and damages heart muscle, liver, adrenals etc.;
- The toxin also cause nerve damage, especially of soft palate(palatine) and eye muscles (ciliary);
- Toxin absorption is negligible in case of skin infection with toxigenic strains;
- Nontoxigenic strains can also produce local disease but systemic effects are absent;

PATHOGENICITY

- Commonest site of infection: Upper respiratory tract (fauces, larynx,nose)
- Ocassionally, other cutaneous or mucocutaneous areas (otitic/conjunctival/ genitovulval/vaginal/ prepucial/skin)
- Faucial diphtheria is the commonest type;
- Sore throat is frequently the presenting symptom;

- After infection, the bacilli multiply on the mucous membrane or skin abrasion;
- The toxigenic strains start producing toxin;
- Diphtheria is a 'toxemia';
- The bacteria confine to the site of entry but the exotoxin is absorbed into the mucus membrane and causes destruction of epithelium and a superficial inflammatory response;

β-prophage (lysogenic bacteriophage)

↓

Encodes diphtheria exotoxin

↓

ADP-ribosylation of elongation factor (EF-2)

↓

Inhibits protein synthesis

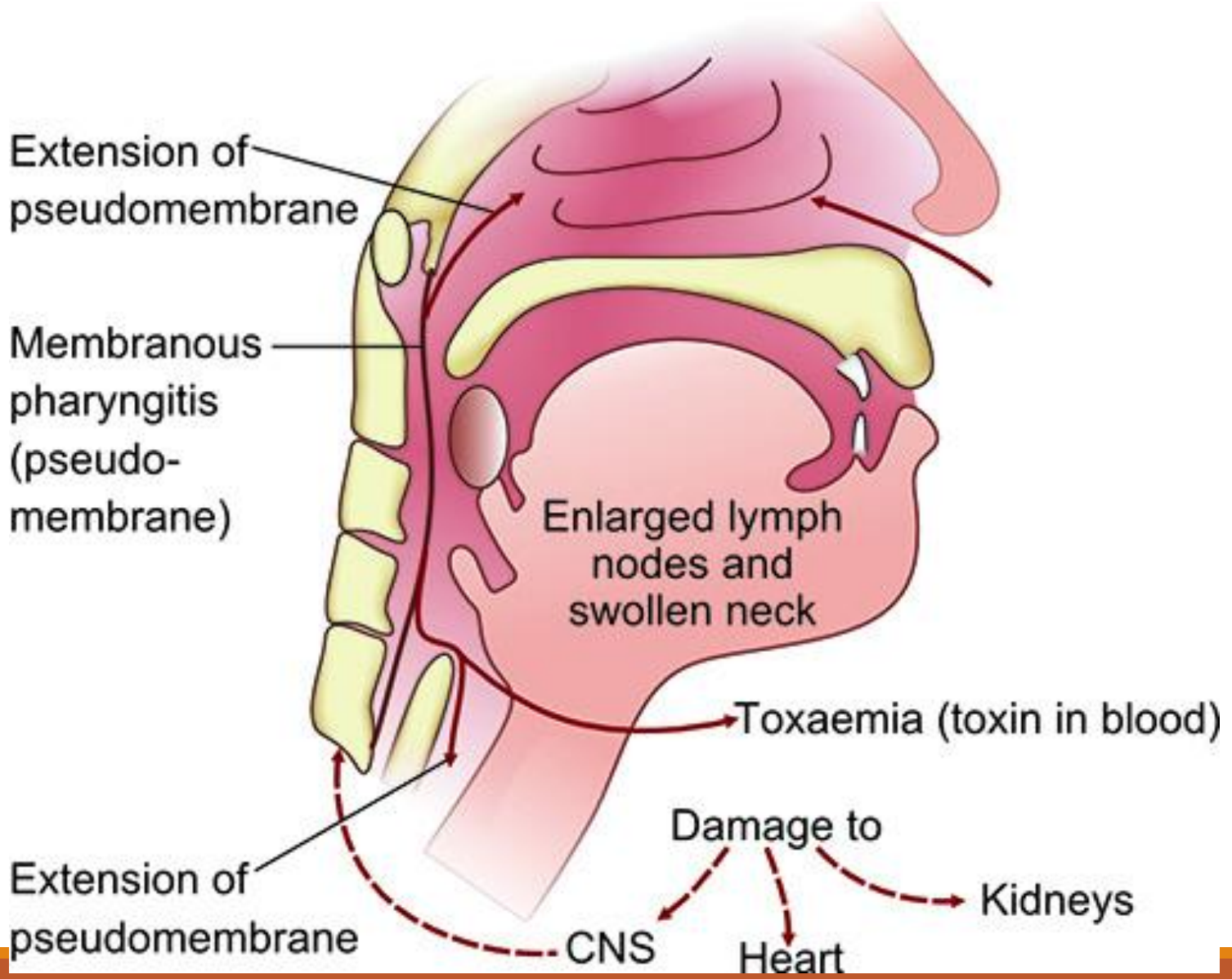
↓

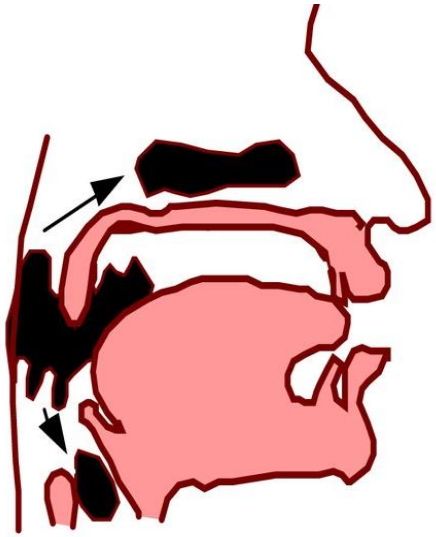
Cell death and clinical manifestations

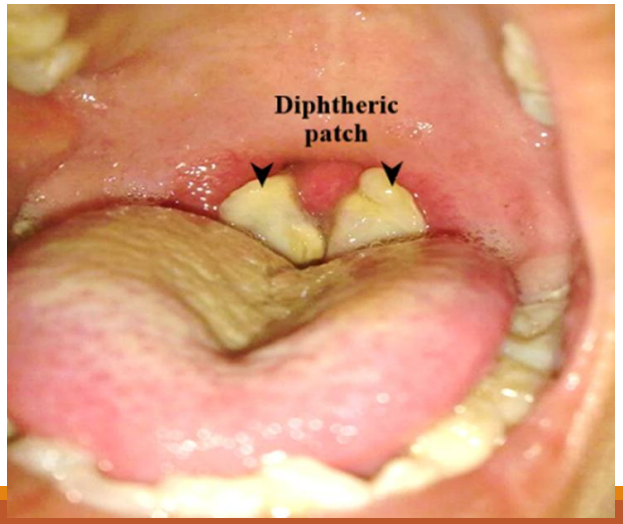
PATHOLOGY

- The toxin causes local necrotic changes;
- The resulting fibrinous exudate, together with the epithelial cells, leucocytes, erythrocytes and bacteria constitute : "pseudomembrane"
- Any effort to remove it will tear off capillaries beneath it and cause bleeding;
- Mechanical complications are due to pseudomembrane and systemic effects are due to the toxin;

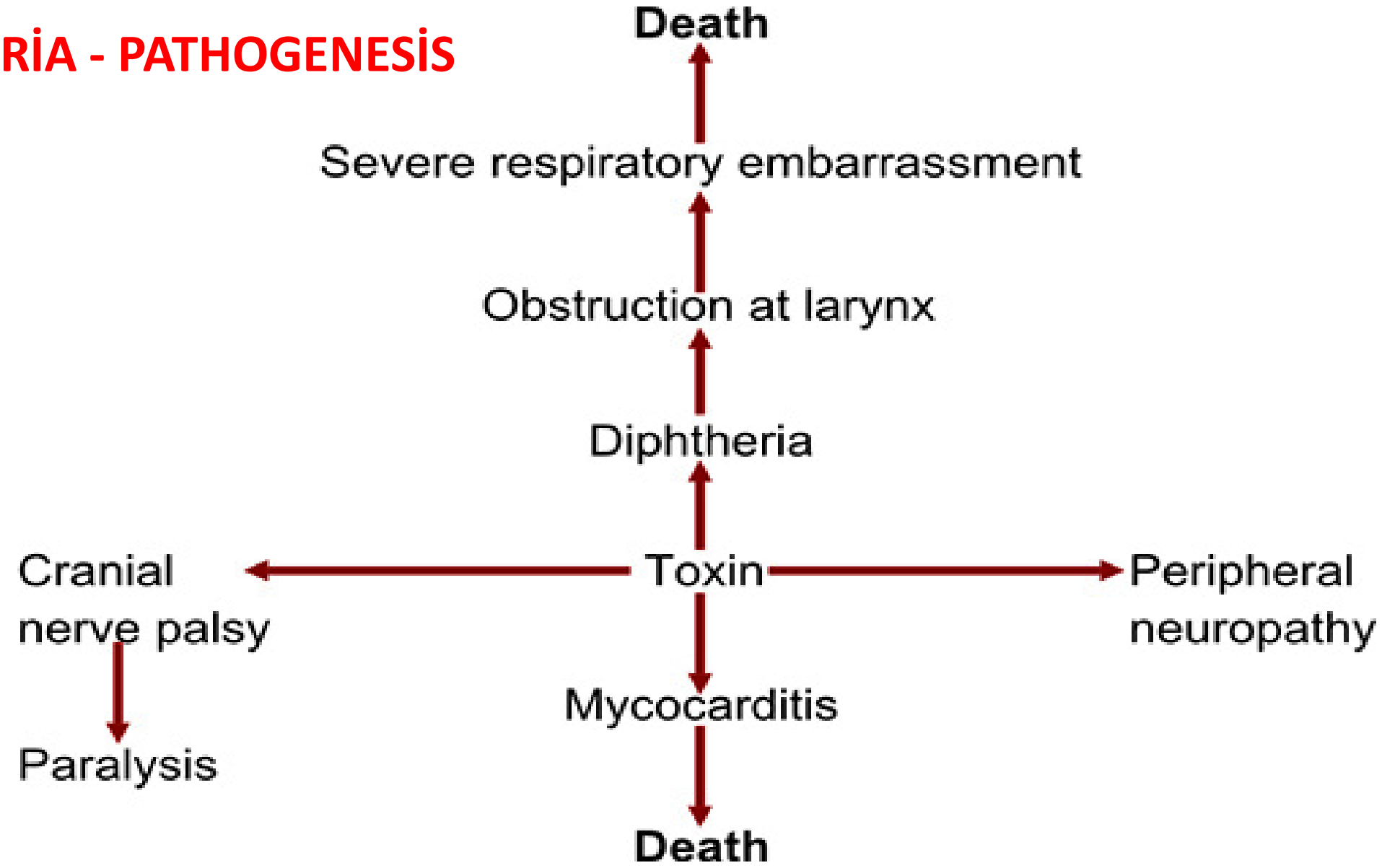
DİPHTHERİA - PATHOGENESİS





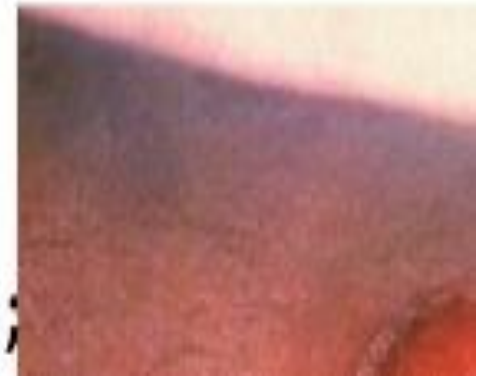


DİPHTHERİA - PATHOGENESİS



CLINICAL DISEASES

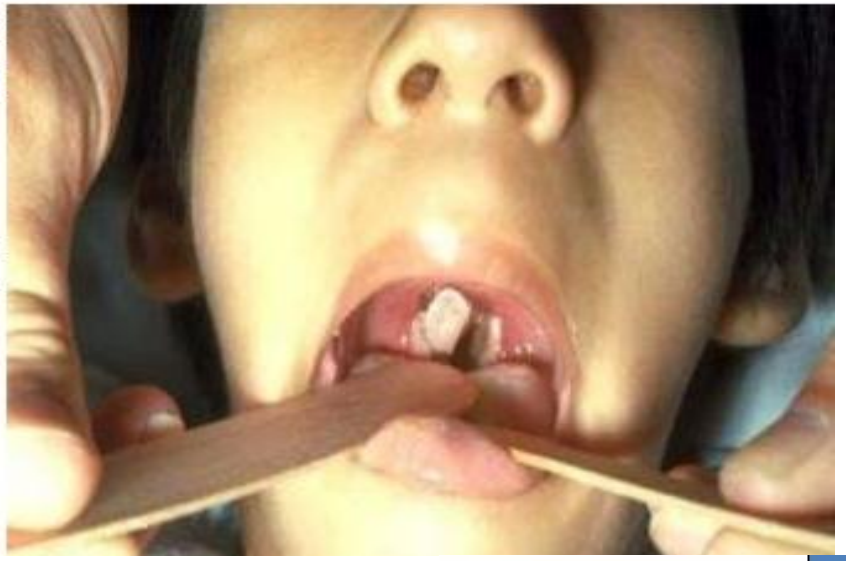
- Incubation period : usually 3-4 days;
- Acute infection : in the form of –
  - **Membranous tonsillitis**
  - **Nasal infection**
  - **Laryngeal infection**
  - **Skin infection –uncommon;**

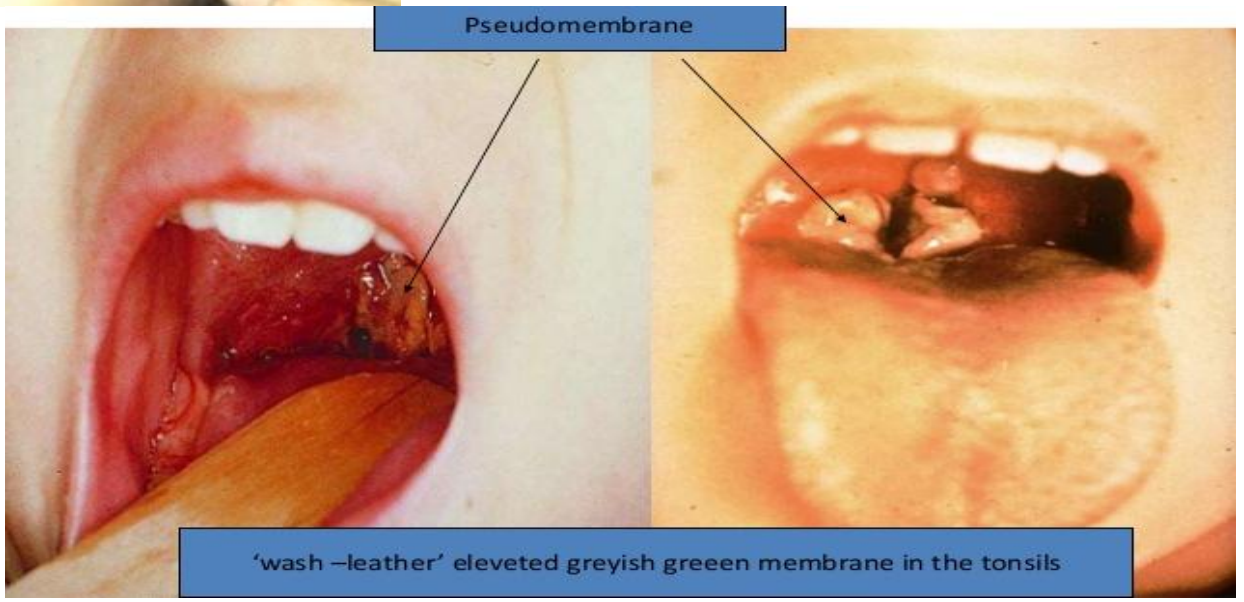


- Nasopharyngeal diphtheria
  - Pharyngeal
  - Laryngeal
- Cutaneous diphtheria
- Systemic complications

CLINICAL DISEASES

- Characteristic feature is : 'wash –leather' eleveted greyish greeen membrane in the tonsils with a well defined edge surrounded by a zone of inflammation;

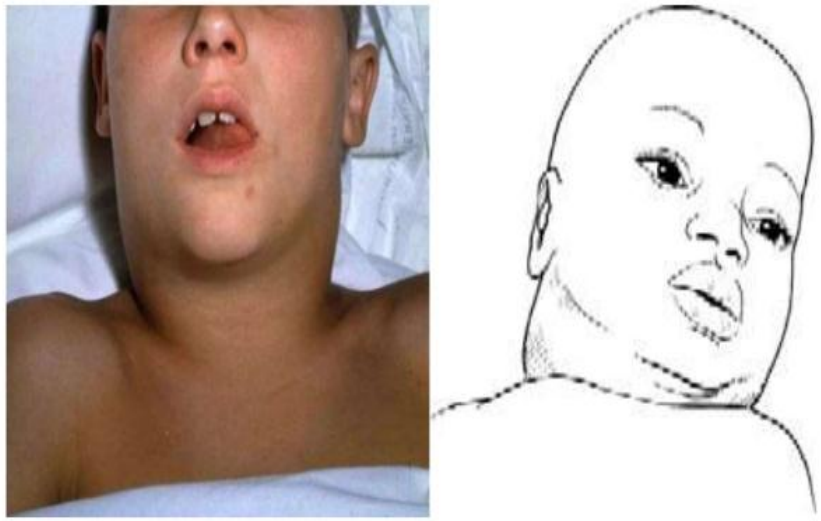




CLASSIFICATION BASED ON CLINICAL SEVERITY

- Malignant or hypertoxic:
  - 'Bull neck' due to marked adenitis in neck;
  - Severe toxemia
  - Circulatory failure
  - Death
  - Paralytic squealae in survivors
- Septic : ulceration, cellulitis and gangrene around pseudomembrane;
- Hemorrhagic: bleeding from the edge of pseudomembrane, epistaxis, purpura etc.

Bull neck : due to cervical adenitis and edema of neck



COMPLICATIONS

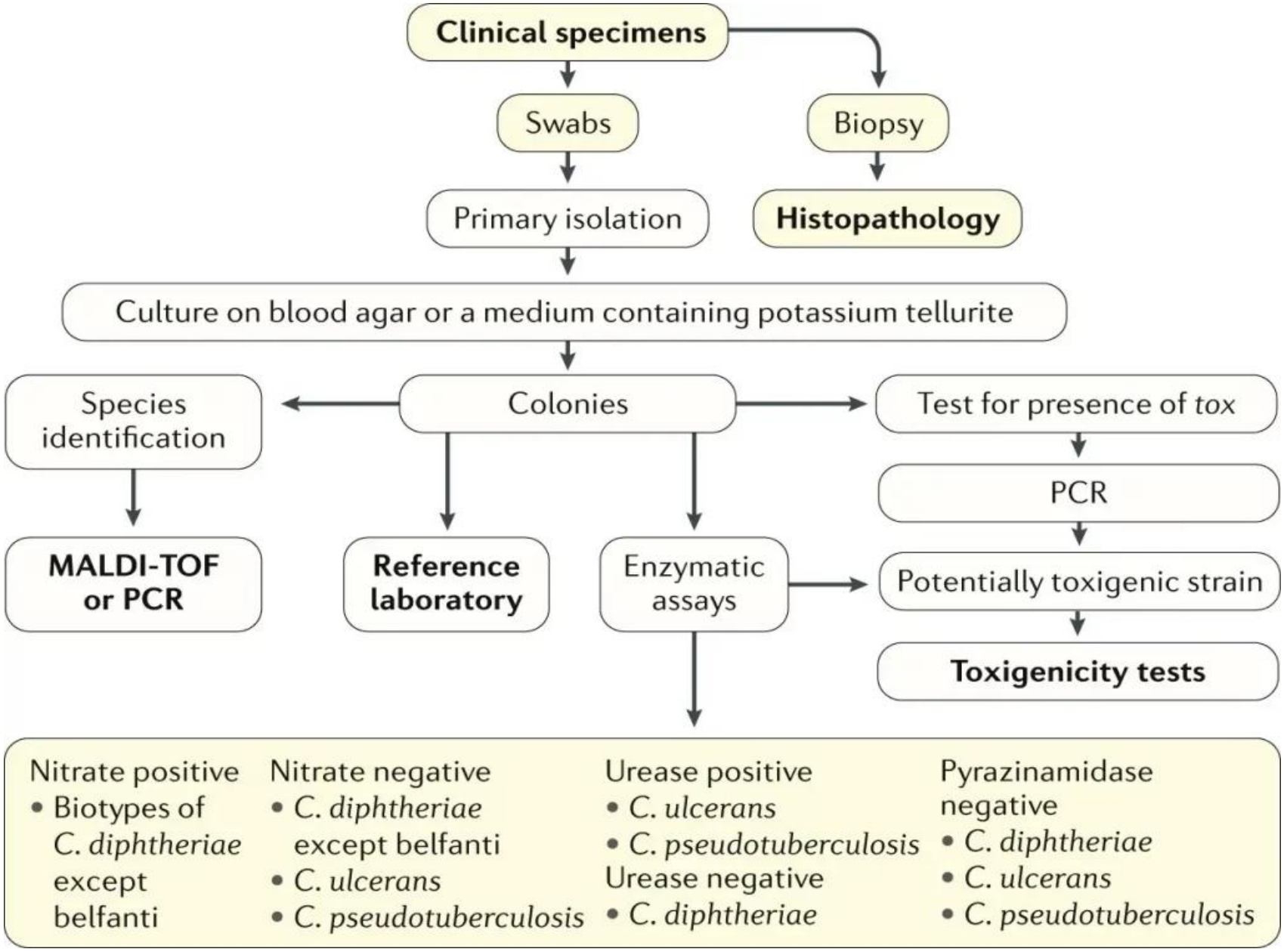
- Asphyxia : due to mechanical obstruction
  - Emergency tracheostomy may be necessary;
- Acute circulatory failure
- Myocarditis
- Postdiphtheritic paralysis-
  - palatine(soft palate) and ciliary (eye muscles) nerves
  - Recovery – spontaneous and complete
- Septic : pneumonia and otitis media
- Relapse : in about 1% of cases

RESISTANCE

- Cultures remain viable for 2-3 wks at 25-30^{0}C
- Destroyed by heat
- Resistant to light, desiccation or freezing;
- Easily destroyed by antiseptics
- Susceptible to – Penicillin, erythromycin and broad spectrum antibiotics;

LABORATORY DIAGNOSIS

- Specimens :
  - Swabs from – nose, throat or other suspected lesions;
- Smear examination: Gram stain
  - shows beaded rods in typical arrangement;
  - Difficult to differentiate from some commensal corynebacteria normally found in throat;
  - Albert's stain or Neisser's stain is useful for demonstrating the granules;


Clinical specimens
Swabs
Biopsy
Primary isolation
Histopathology
Culture on blood agar or a medium containing potassium tellurite
Species identification
Colonies
Test for presence of tox
PCR
MALDI-TOF or PCR
Reference laboratory
Enzymatic assays
Potentially toxigenic strain
Toxigenicity tests
Nitrate positive
• Biotypes of C. diphtheriae except belfanti
Nitrate negative
• C. diphtheriae except belfanti
• C. ulcerans
• C. pseudotuberculosis
Urease positive
• C. ulcerans
• C. pseudotuberculosis
Urease negative
• C. diphtheriae
Pyrazinamidase negative
• C. diphtheriae
• C. ulcerans
• C. pseudotuberculosis

LABORATORY DIAGNOSIS

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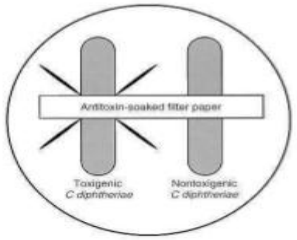
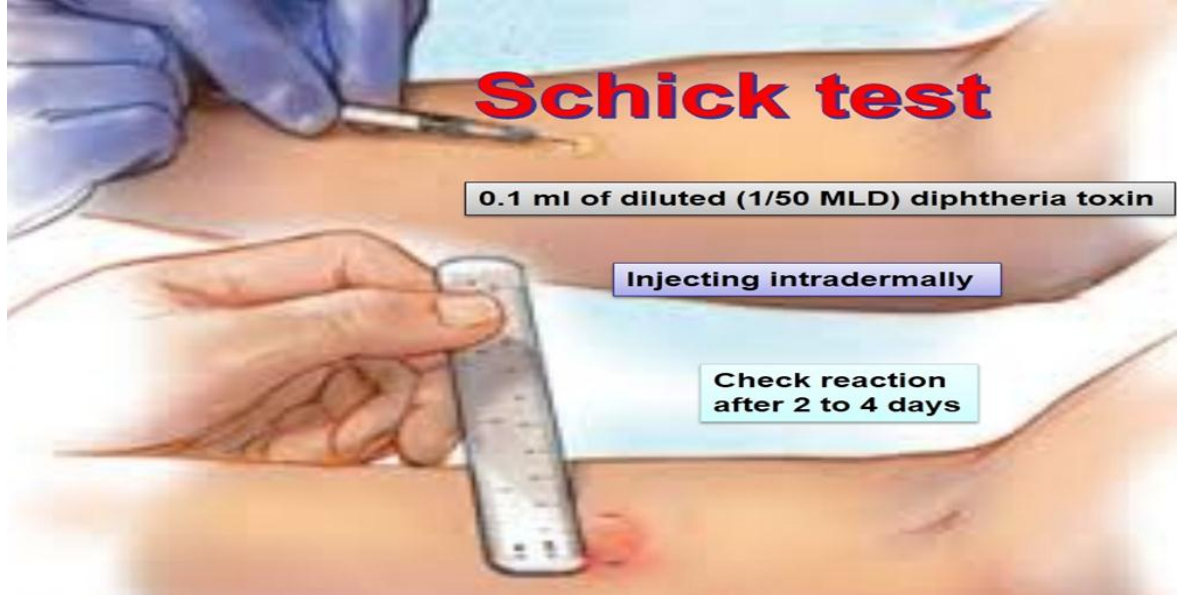
Elek immunodiffusion test

- A sterile, antitoxin-saturated filter paper strip is embedded in the culture medium, and *C diphtheriae* isolates are streak-inoculated at a 90° angle to the filter paper.
- The production of diphtheria toxin can be detected within 18 to 48 hours by the formation of a toxin-antitoxin precipitin band in the agar.



Sterile filter paper impregnated with diphtheria antitoxin is imbedded in agar culture medium. Isolates of *C diphtheriae* are then streaked across the plate at an angle of 90° to the antitoxin strip. Toxigenic *C diphtheriae* is detected because secreted toxin diffuses from the area of growth and reacts with antitoxin to form lines of precipitin.



LABORATORY DIAGNOSIS : CULTURE

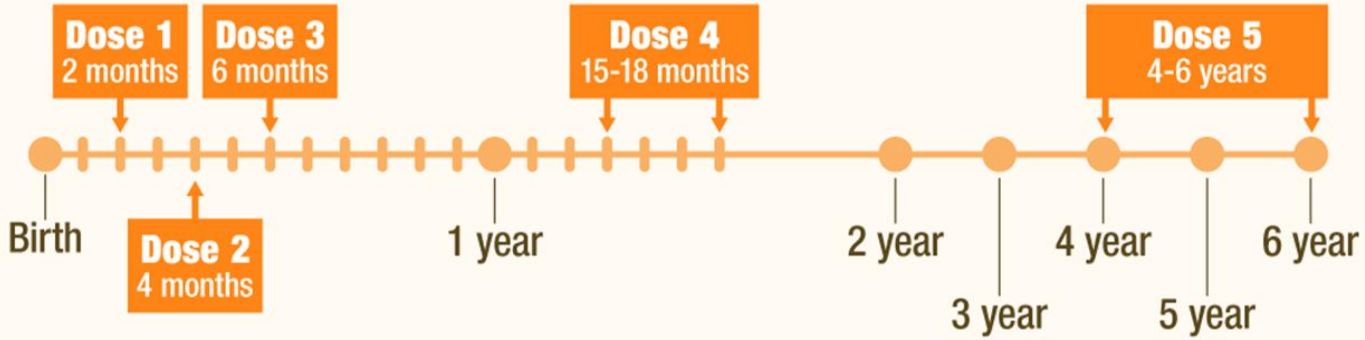
- If the swabs can not be inoculated promptly, they should be kept moistened with serum;
- Inoculate on :
  - – **Loeffler's serum slope**
  - – **Tellurite blood agar or Tinsdale medium**
  - – **Blood agar (for differentiating Staphylococcal or Streptococcal pharyngitis that simulate diphtheria);**
- *Tellurite medium is particulary useful for isolating the organism from – convalescents, contacts or carriers;*

Prevention

Vaccination: Immunisation with diphtheria toxoid, combined with tetanus and pertussis toxoid (DTP vaccine), should be given to all children at two, three and four months of age. Booster doses are given between the ages of 3 and 5 .

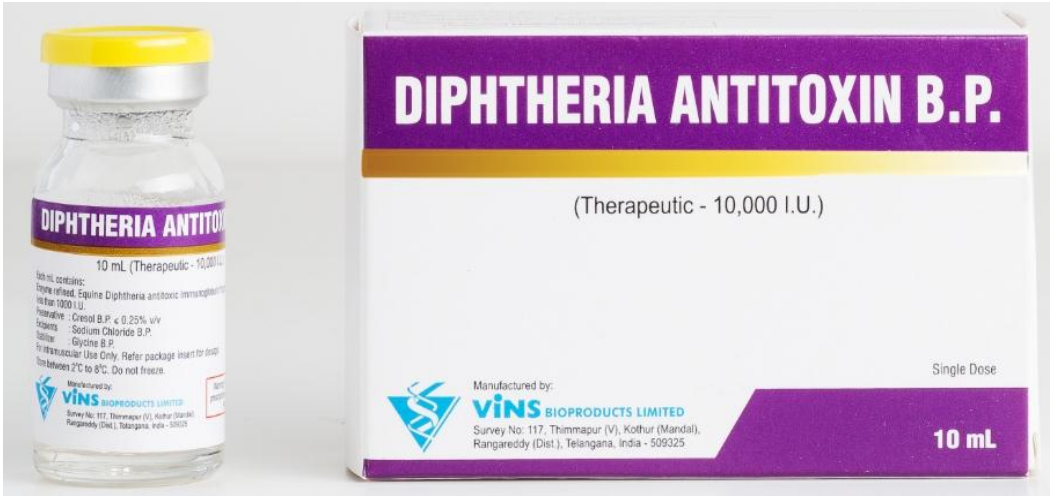
The child is given a further booster vaccine before leaving school and is then considered to be protected for a further 10 years (16 – 18 years).

DTaP: Diphtheria, Tetanus, and Acellular Pertussis



Treatment

- Antibiotic not useful in Acute infections
- Antitoxin a must.
- Anti toxin obtained from horse serum
- Mild 20,000 to 40,000
- Moderate 40,000 to 60,000
- Severe 80,000 to 1,00,000
- Commonly used antibiotics,
- Penicillin parentally,
- Oral Erythromycin



Bordetella - Taxonomy

(Domain): Bacteria

(Kingdom): Pseudomonadota

(Class): Betaproteobacteria

(Order): Burkholderiales

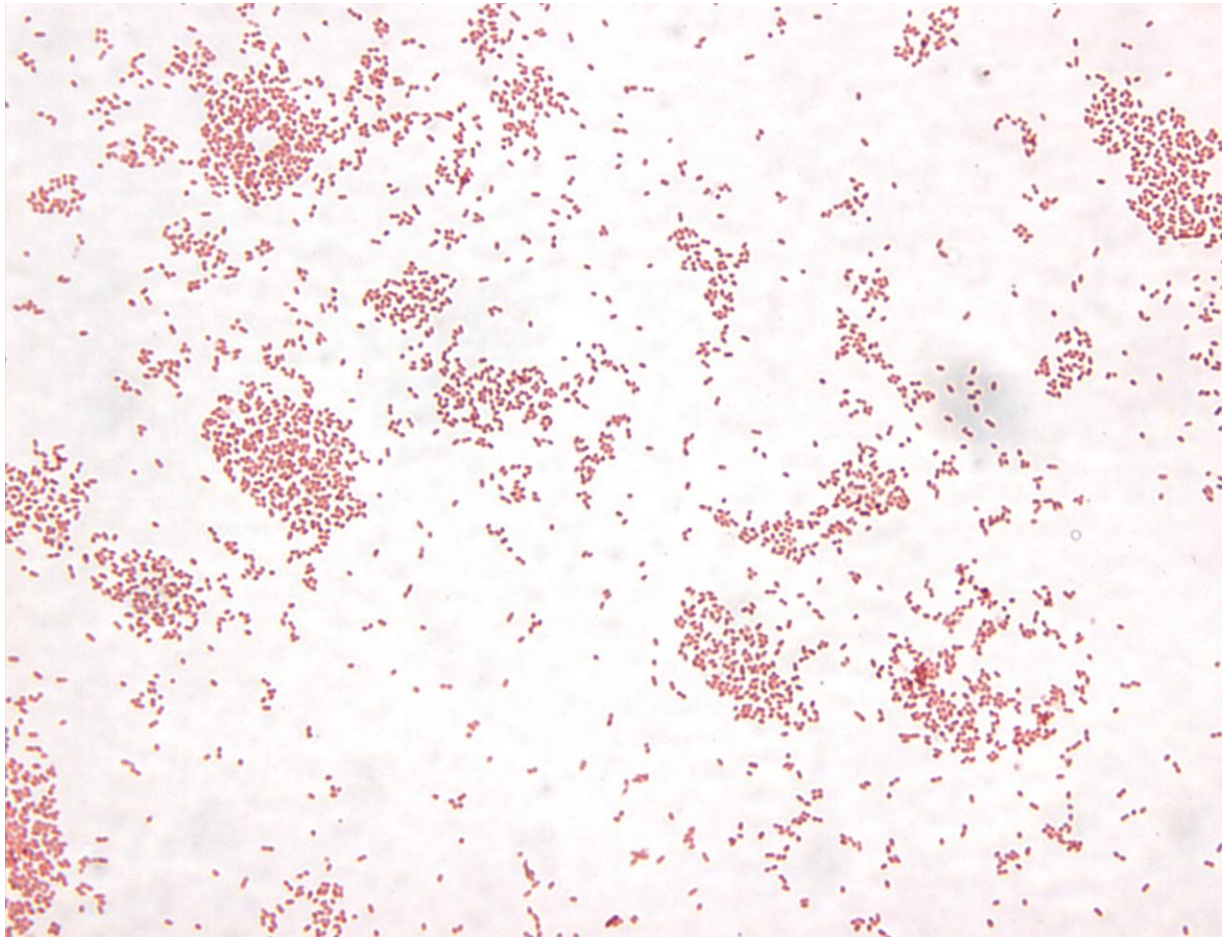
(Family): Alcaligenaceae

(Genus): Bordetella

(Species): ***B.pertussis, B.parapertussis,*** *B.bronchiseptica, B.avium etc.*

Morphology

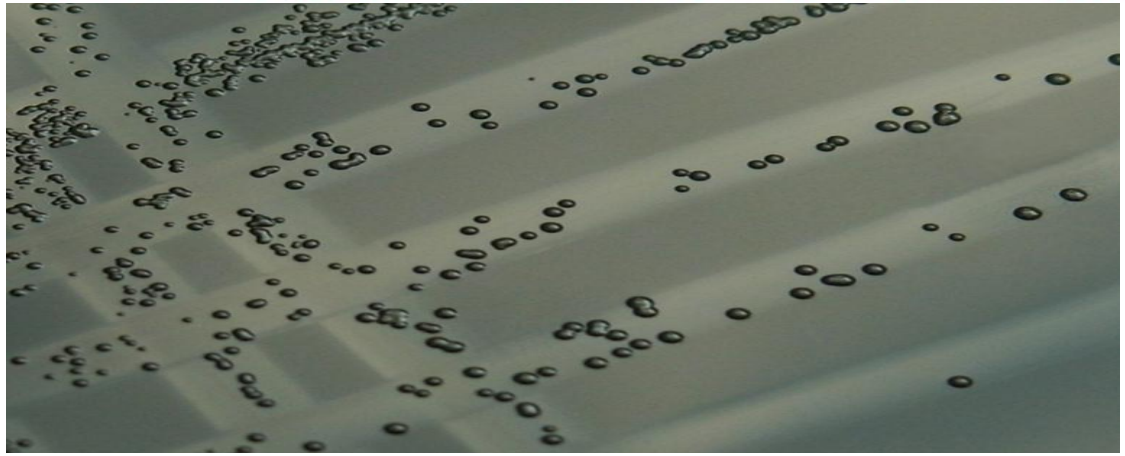
- It is Gram negative.
- It is a small, ovoid coccobacillus (mean length 0.5 µm).
- It is nonmotile and nonsporing.
- It is capsulated.
- Freshly isolated strains of Bord pertussis have fimbriae.



Colonies on Bordet-Gengou medium

- Growth is slow(48-72 hours).
- Colonies are small, dome-shaped, smooth, opaque, viscid, greyish white, refractile and glistening, resembling 'bisected pearls' or 'mercury drops'.
- Surrounded by a hazy zone of hemolysis.
- Confluent growth presents an 'aluminium paint' appearance.

- Aerobic, Not anaerobic
- Grows optimally at 35^0 to 37^0 c
- Complex media are necessary for primary isolation
- Preferred medium – **Bordet Gengou glycerin potato blood agar**
- Blood for neutralizing inhibitory substances formed during bacterial growth.
- Charcoal also serves the same purpose.
- Charcoal blood agar is a useful medium.
- It does not grow on, simple media like nutrient agar.



Differentiating features of Bordetella species

	B.pertusis	B.Parapert - usis	B.bronchis eptila	B.canium
Motality	-	-	+	+
Growth on nutrient agar	-	+	+	+
Growth B-G medium	3 - 6	1 -2	1	1
urease	-	+	+	-
Nitrate to nitrate	-	-	+	-

Biochemical reactions

Do not ferment sugars

Indole test +

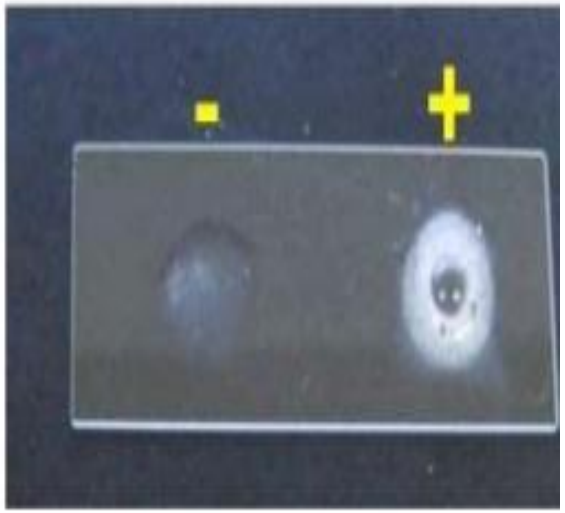
Nitrates +

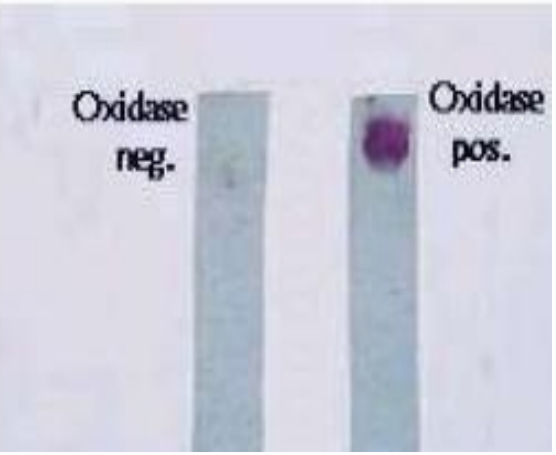
Citrates +

Urease +

Catalase +

Oxidase +



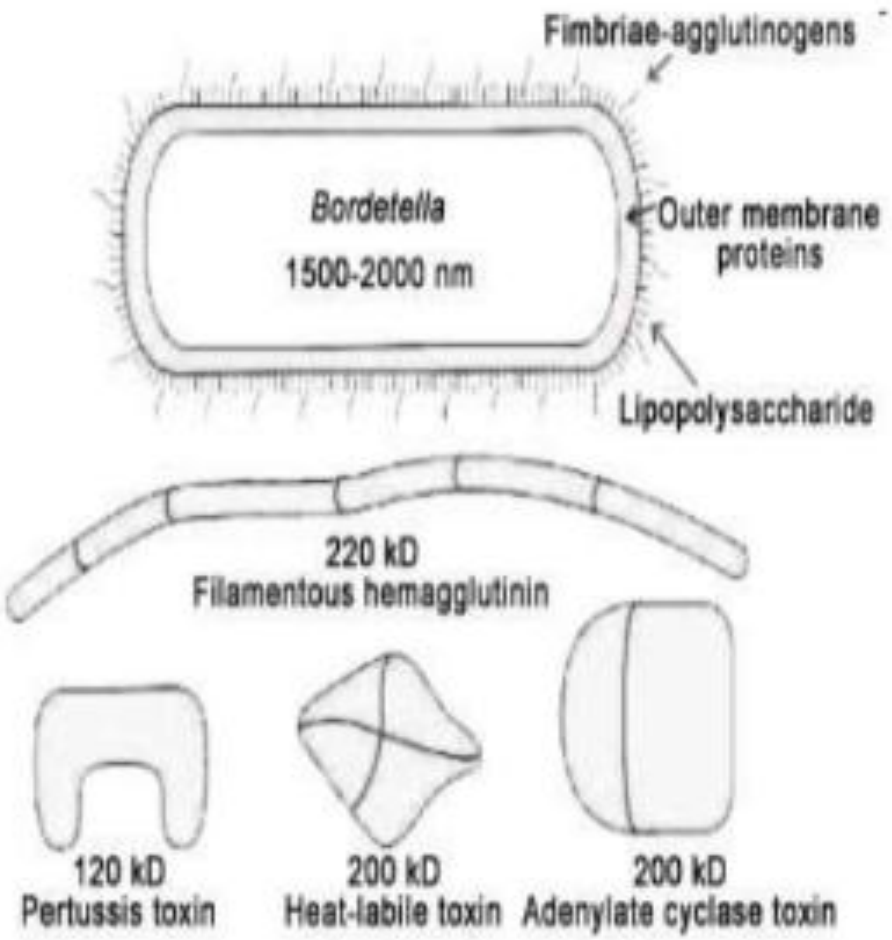


Resistance

- It is a delicate organism, being killed readily by heat (55°C for 30 minutes), drying and disinfectants.
- Outside the body, Bord pertussis in dried droplets is said to survive for five days on glass, three days on cloth and a few hours on paper.

Antigenic constituents and virulence factors

- Several antigenic fractions and putative virulence factors have been described but their role in the pathogenesis of pertussis remains to be clarified.
- These virulence factors include:
- Adhesions such as filamentous hemagglutinin, agglutinogens, peractin, and fimbriae.
- A number of toxins including pertussis toxin, acetylate cyclase toxin, trachael cytotoxins, Dermonecrtoic toxin and heat-labile toxin.



Agglutinogens

- Species specific surface agglutinogens with capsule K antigens or fimbria
- 14 agglutinin factors are identified
- Factors 7 is common in all species
- Factor 1- 6 in only B pertussis
- Factor 12 in B.brochoseptica
- Factor 14 in B parapertussis
- Agglutinogens promote virulence by helping bacteria to attach to respiratory epithelial cells.
- They are useful in serotyping strains and in epidemiological studies.

Virulence factors of *Bordetella pertussis*

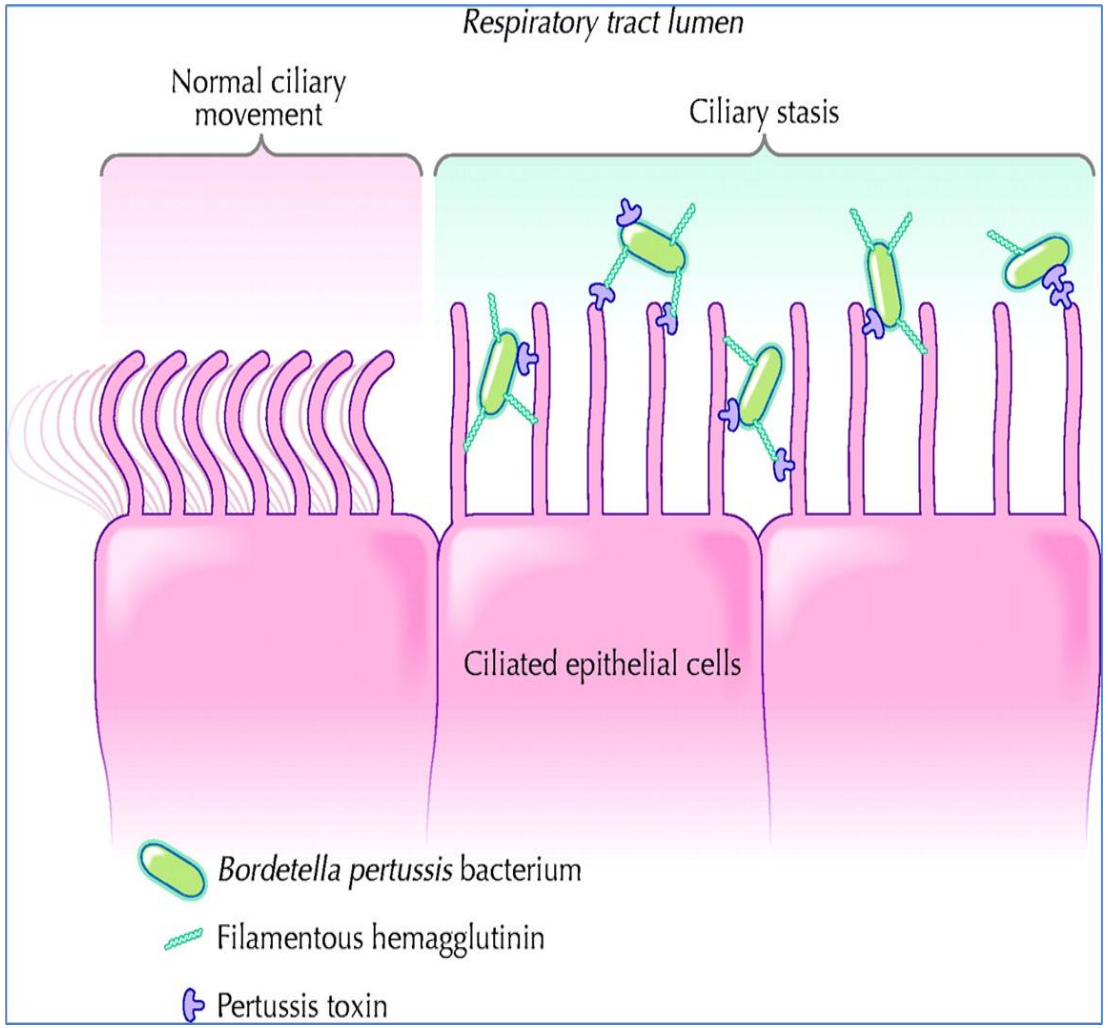
Virulence factors	Biological functions
Filamentous hemagglutinin	Binds to galactose residues on a sulfated glycolipid called sulfatide, present on the surface of the ciliated cells; binds to CR3, a receptor on the surface of polymorphonuclear leukocytes; and mediates the attachment of *Bordetella pertussis* to ciliated epithelial cells of the respiratory tract
Pertussis toxin	Causes adhesion of *Bordetella pertussis* to tracheal epithelium; S2 subunit binds to glycolipid present on ciliated epithelium; S3 subunit binds to ganglioside receptor on the surface of phagocytic cells; and S1 subunit inhibits the eukaryotic adenyl cyclase, killing of phagocytes, and migration of monocytes
Invasive adenylate cyclase or hemolysin	It has adenylate cyclase activity and a binding component that mediates attachment to host cell surface
Lethal toxin	Causes inflammation and lethal necrosis around the site of adherence of the bacteria
Tracheal cytotoxin	Kills ciliated respiratory cells; also stimulates release of cytokine IL-1 and inhibits ciliary movement

Pertussis toxin (PT)

- This is present **only in Bord pertussis**. It plays an important role in the pathogenesis of **whooping cough**.
- PT is expressed on the surface of the bacillus and secreted into the surrounding medium
- The toxin exhibits diverse biological and biochemical activities, which formerly had been believed to be caused by different substances that had been named accordingly.
- Examples are the lymphocytosis producing factor(LPF), causing profound lymphocytosis in pertussis patients as well as in experimental animals, and two effects seen only in experimental animals but not in patients, Cont.....

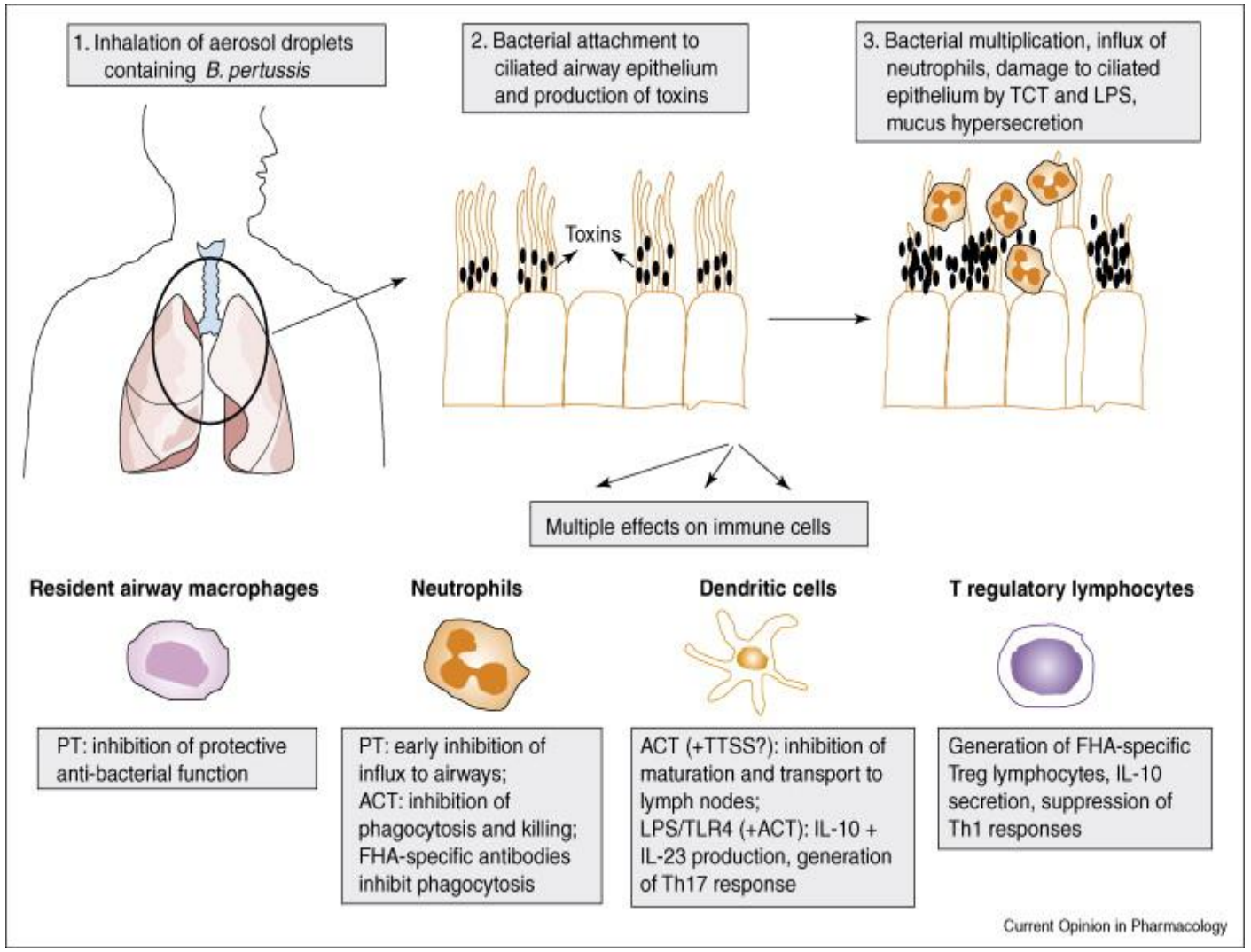
Pertussis toxin (PT)

- Such as the histamine sensitising factor(HSF) responsible for heightened sensitivity to histamine in experimental animals, and the islet activating protein(IAP) inducing excessive insulin secretion by the pancreatic islet cells.
- It is now known that all these are manifestations of the pertussis toxin
- PT is a 117,000 molecular weight hexamer protein composed of six subunits with an A-B structure (the A portion being the enzymatically active moiety and B the binding component).
- **It can be toxoided**.
- PT toxoid is the major component of acellular pertussis vaccines

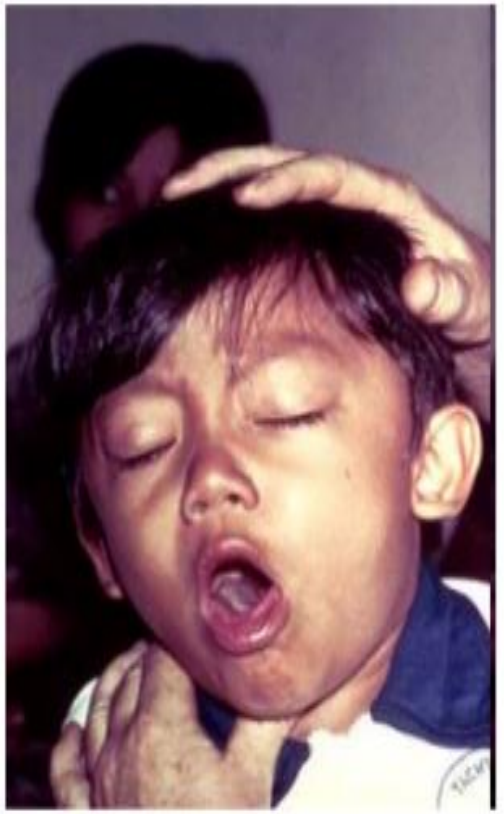


Pathogenicity & Clinical Manifestations

- In human beings, after an incubation period of about 1-2 weeks, the disease takes a protracted course comprising three stages:
- the catarrhal,
- paroxysmal and
- convalescent each lasting approximately two weeks.
- The onset is insidious, with low grade fever, catarrhal symptoms and a dry, irritating cough.
- Clinical diagnosis in the catarrhal stage is difficult.
- This is unfortunate as this is the stage at which the disease can be arrested by antibiotic treatment.


1. Inhalation of aerosol droplets containing *B. pertussis*
2. Bacterial attachment to ciliated airway epithelium and production of toxins
3. Bacterial multiplication, influx of neutrophils, damage to ciliated epithelium by TCT and LPS, mucus hypersecretion
Toxins
Multiple effects on immune cells
Resident airway macrophages
Neutrophils
Dendritic cells
T regulatory lymphocytes
PT: inhibition of protective anti-bacterial function
PT: early inhibition of influx to airways; ACT: inhibition of phagocytosis and killing; FHA-specific antibodies inhibit phagocytosis
ACT (+TTSS?): inhibition of maturation and transport to lymph nodes; LPS/TLR4 (+ACT): IL-10 + IL-23 production, generation of Th17 response
Generation of FHA-specific Treg lymphocytes, IL-10 secretion, suppression of Th1 responses
Current Opinion in Pharmacology

Bordetella

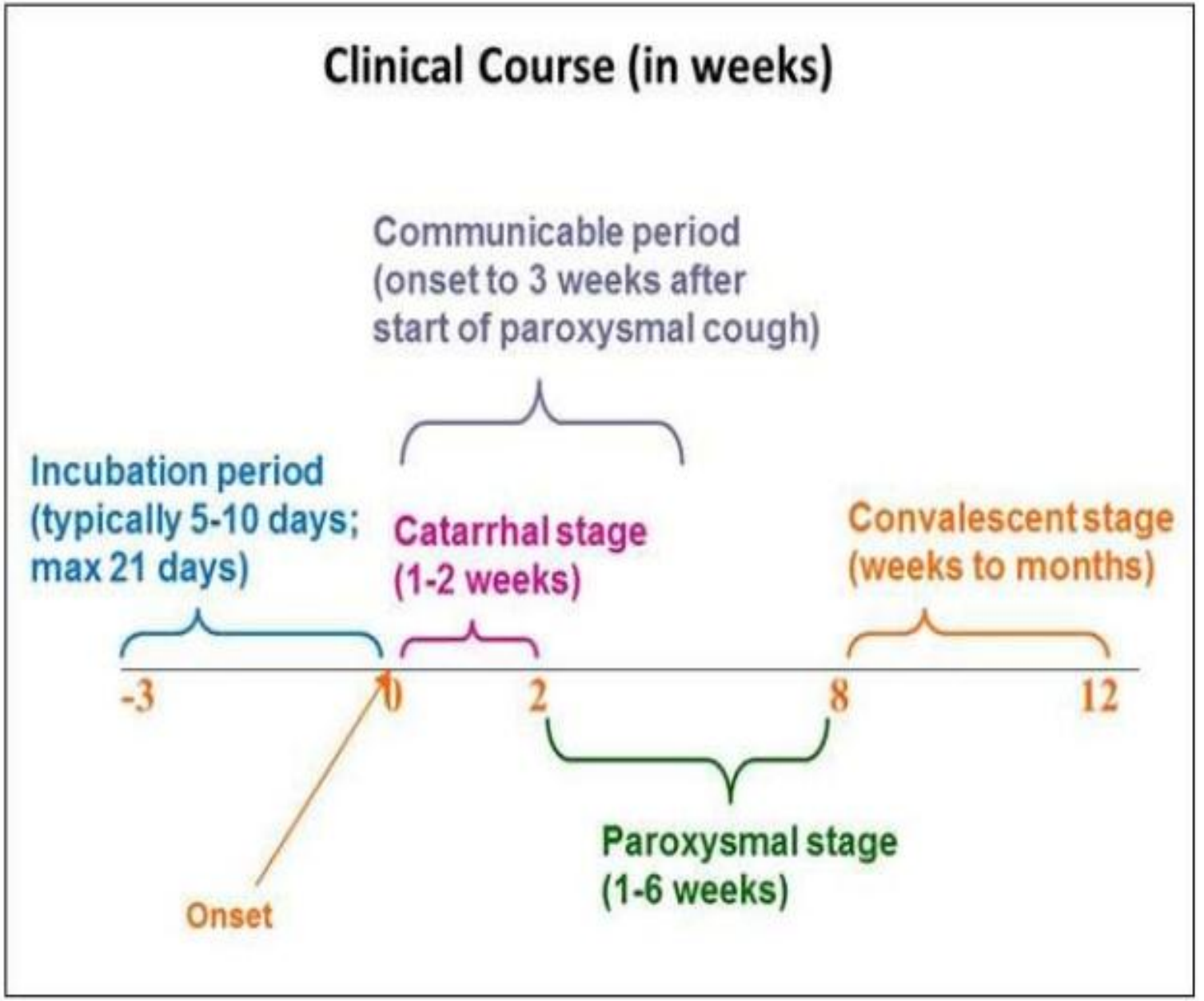


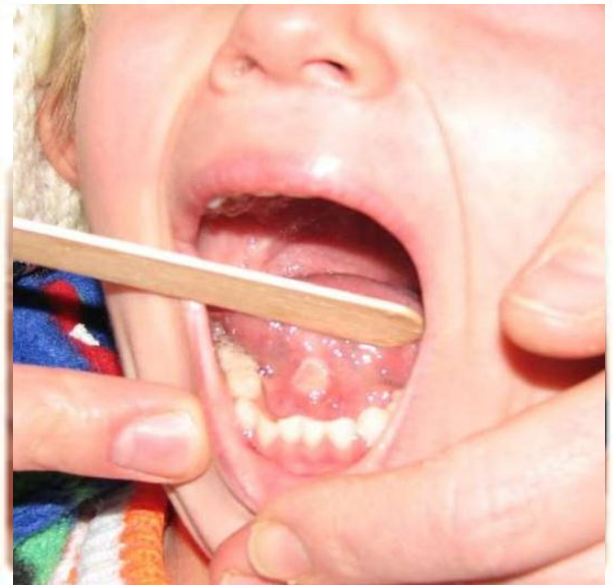
Clinical Manifestations of Pertussis

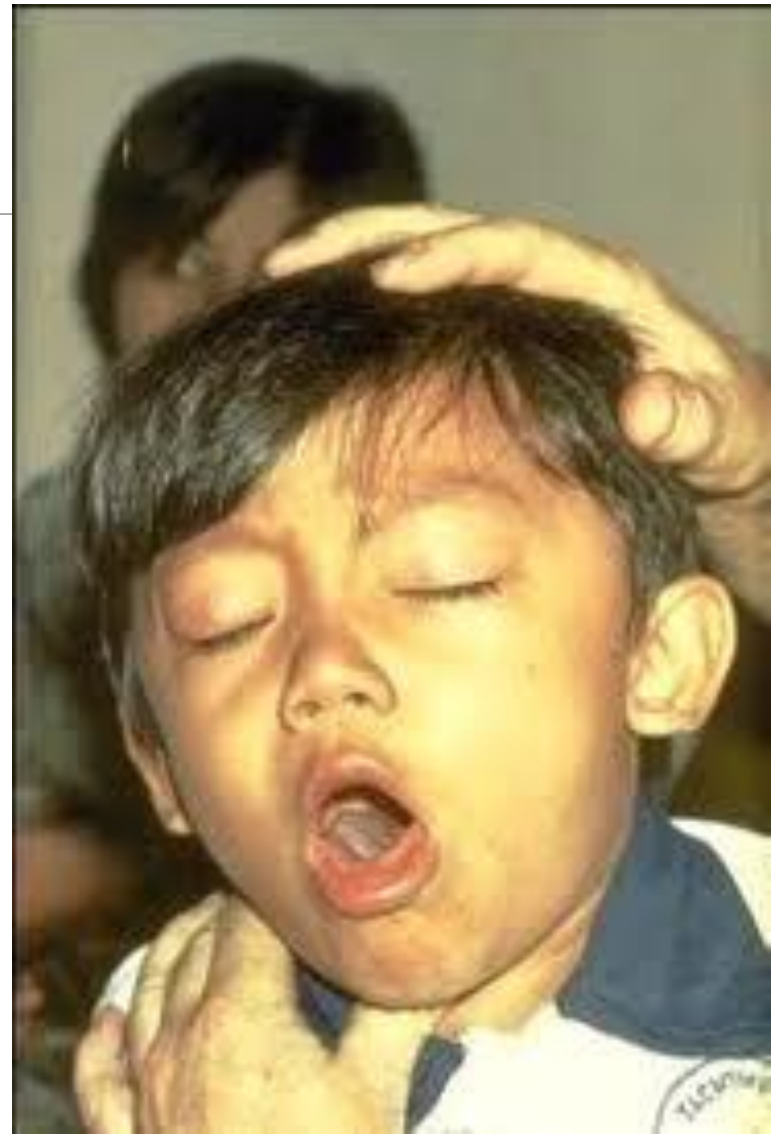
- Incubation period 3-12 days (up to 21 days)
- Insidious onset, similar to minor upper respiratory infection with nonspecific cough
- Fever usually minimal throughout course
- Apnea & Cyanosis in infant

STAGES

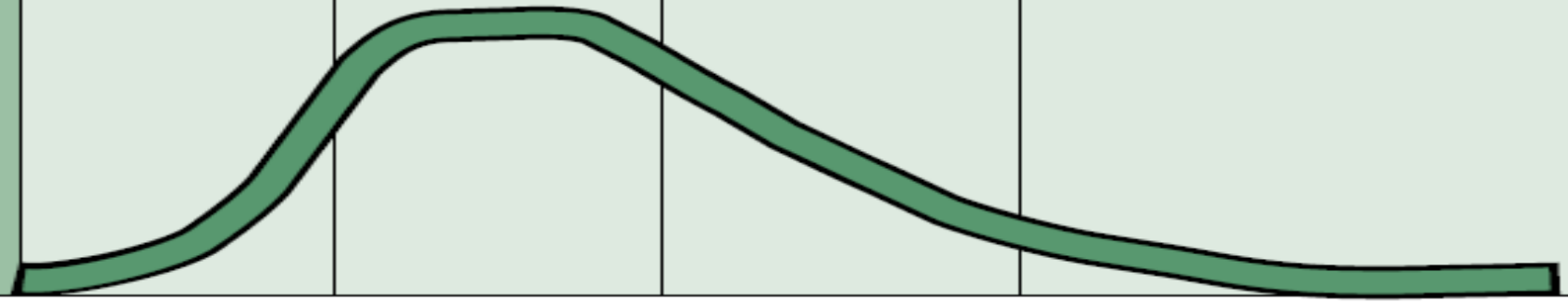
- 1st Stage- Catarrhal Stage
- 2nd Stage- Paroxysmal Stage
- 3rd Stage- Covalescent Stage





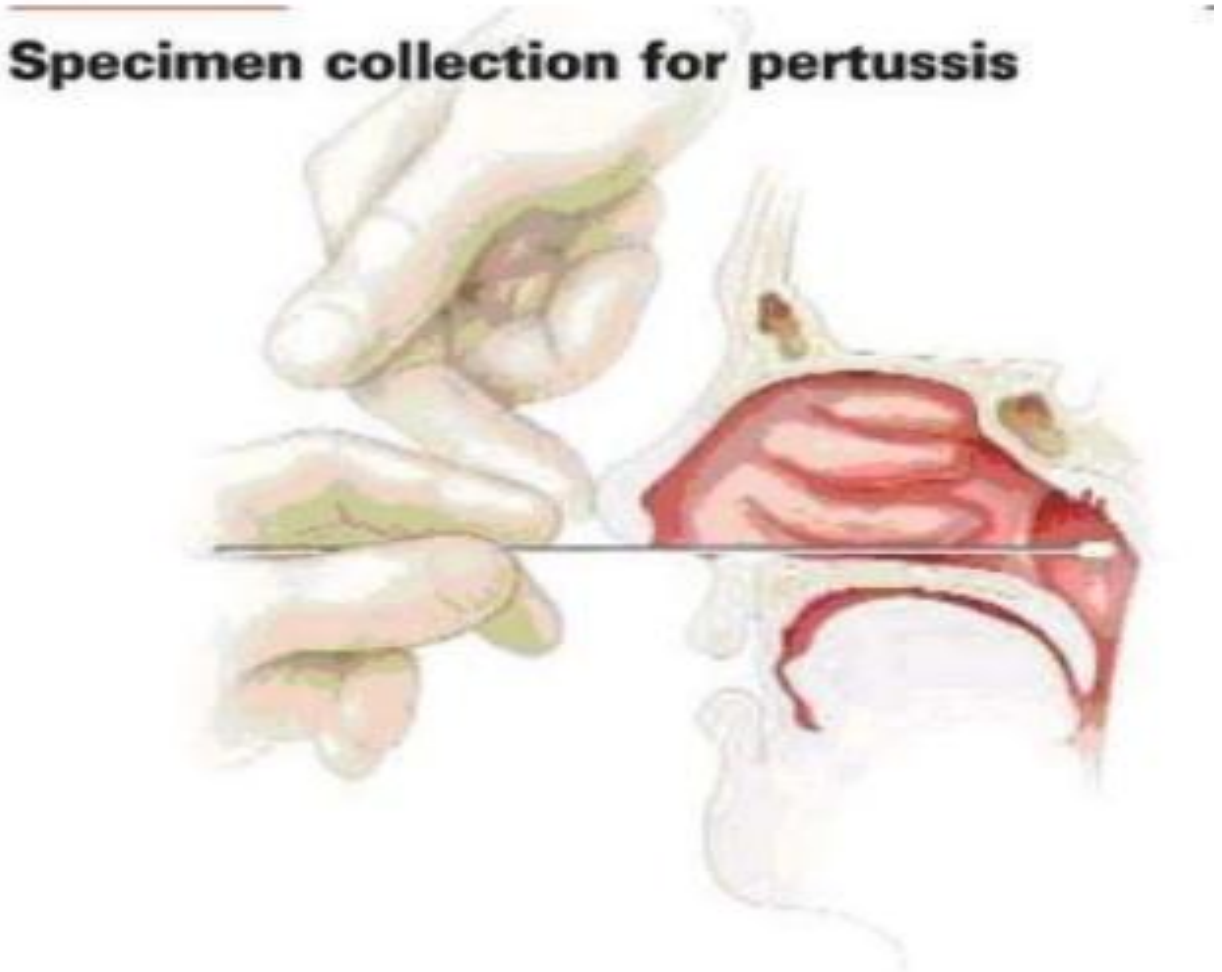


Clinical presentation of *Bordetella pertussis* disease.

	Incubation	Catarrhal	Paroxysmal	Convalescent
Duration	7-10 days	1-2 weeks	2-4 weeks	3-4 weeks (or longer)
Symptoms	None	Rhinorrhea, malaise, fever, sneezing, anorexia	Repetitive cough with whoops, vomiting, leukocytosis	Diminished paroxysmal cough, development of secondary complications (pneumonia, seizures, encephalopathy)
Bacterial culture				

Nasopharyngeal Swab

- Secretion from the posterior pharyngeal wall are collected with cotton swab on a bent wire passed from the oral cavity
- A West's post nasal swab is used for collection of specimen.



Laboratory Diagnosis

- Microscopy – Demonstration of Bacilli in respiratory secretions.
- Florescent Antibody methods.
- Specimen Collection:
- Cough Plate Method:
- Culture plate held at 10-15 cm infront of the mouth when the patient is coughing spontaneously or induced cough
- Droplets of respiratory exhaled impinge on the media.
- Helpful as bed side investigation

Cough Plate Method

Serology

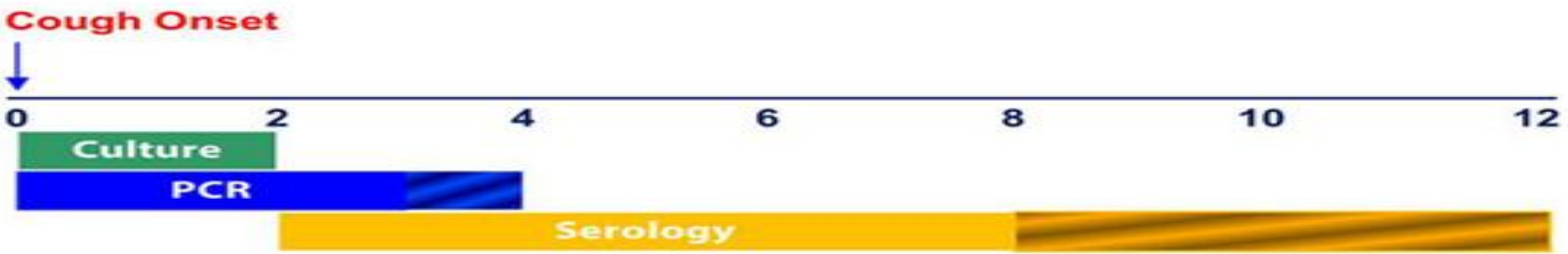
Paired serum sample for detection of antibodies

Gel precipitation testing

Complement fixation test

Detection of Ig A by ELISA from nasopharyngeal secretions.

Optimal Timing for Diagnostic Testing (weeks)



Prophylaxis

Preventing the spread of infection by isolation of cases is seldom practicable, as infectivity is highest in the earliest stage of the disease when clinical diagnosis is not easy.

Specific immunisation with killed Bord pertussis vaccine has been found very effective.

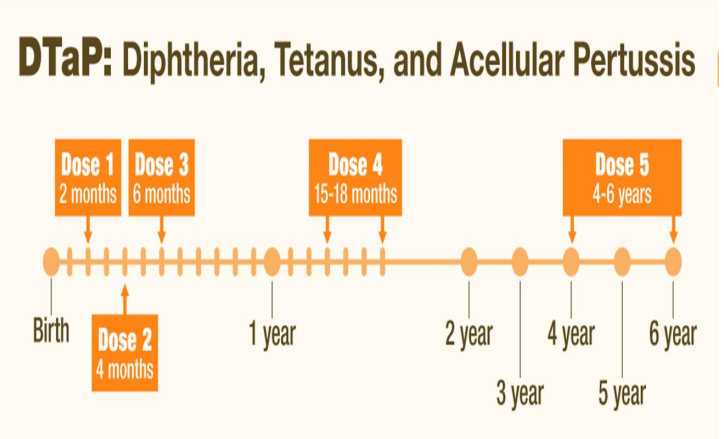
The alum absorbed vaccine produces better and more sustained protection and less reaction than the plain vaccines.

Pertussis vaccine is usually administered in combination with diphtheria and tetanus toxoid (triple vaccine)(DTwP/DTaP).

Not only is this more convenient but Bord pertussis also acts as an adjuvant for the toxoids, producing better antibody response.

Choice of regimen

- Erythromycin
- Azithromycin
- Clarithromycin



Haemophilus

Scientific classification

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gamma Proteobacteria
Order: Pasteurellales
Family: Pasteurellaceae
Genus: Haemophilus
Species: influenzae
Binomial name: *Haemophilus influenzae*

Haemophilus species of clinical importance

1. ***H. influenzae***

-**type b is an important human pathogen**

2. ***H. ducreyi***

-**sexually transmitted pathogen (chancroid)**

3. Other *Haemophilus are normal flora*

- *H. parainfluenzae – Pneumonia & endocarditis*
- *H. aphrophilus – Pneumonia & endocarditis*
- *H. aegyptius – Pink eye (purulent conjunctivitis)*

Haemophilus Influenza

Morphology

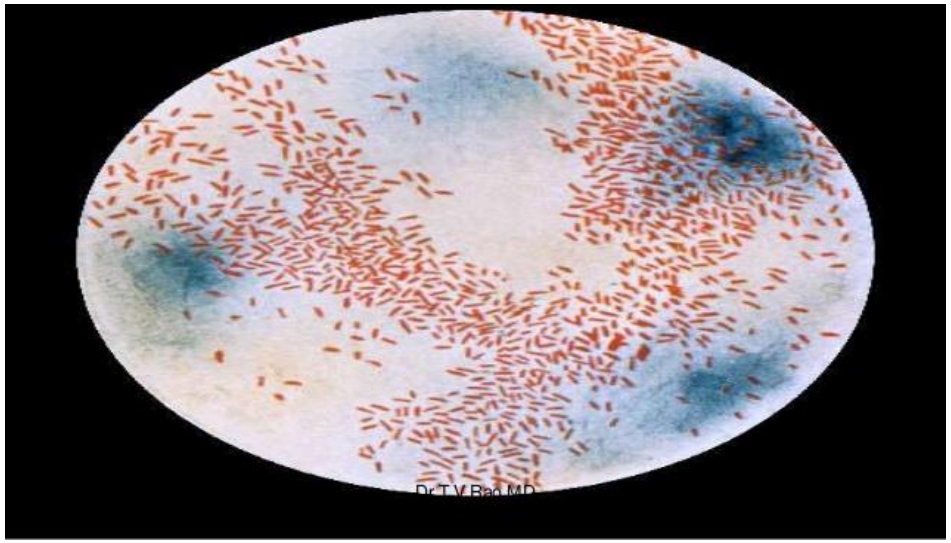
- Size is (1-2 X 0.3 – 0.5 microns)
- Non motile,
- Non sporing
- Gram negative rod or coccobacillus
- Pleomorphic (old culture)
- Appear as clusters of Coccobacillary forms in infected Sputum
- Long bacillary and filamentous form in infected CSF (Meningitis)

Aerobic gram-negative bacteria

Polysaccharide capsule

Six different serotypes (a-f) of polysaccharide capsule

95% of invasive disease caused by type b (Hib)



Gram staining

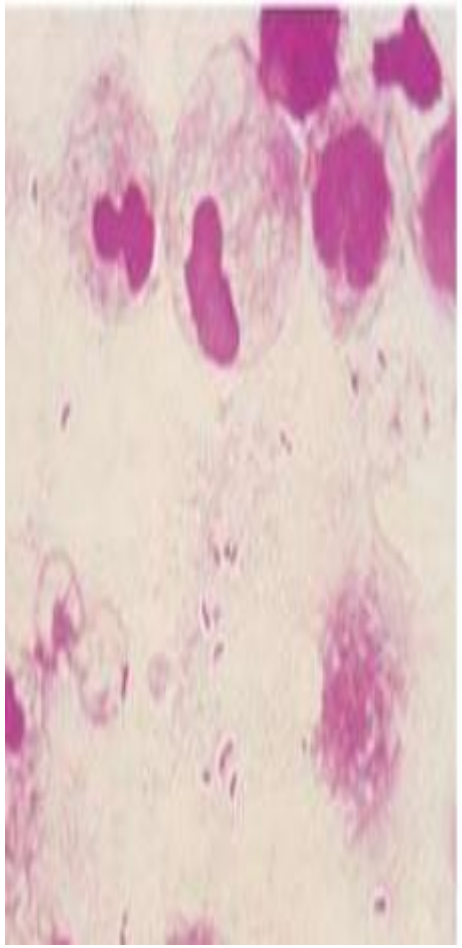


Fig: Gram-stained CSF sediment preparation.
Fine, Gram-negative rods surrounded by a capsule (serovar b).
Clinical diagnosis: purulent meningitis

Culture characteristics

Fastidious growth requirements

Factors X and V are essential for growth

X is Hemin heat stable

Porphyrins for synthesis of Cytochromes

V-factor (NAD): Heat- labile, coenzyme I, nicotinamide adenine dinucleotide, found in blood – oxidation

Aerobic 37 dg C

Haemophilus Species, cont.

Species	X	V	Hemolysis
►H. influenzae (H. aegyptius)	+	+	-
►H. parainfluenzae	-	+	-
►H. ducreyi	+	-	-
►H. haemolyticus	+	+	+
►H. parahaemolyticus	-	+	+
►H. aphrophilus	-	-	-

Culture characteristic

On Chocolate agar, flat, grayish-brown colonies, 1-2 mm in diameter present after 24 hrs

Colonies of staphylococci on sheep Blood agar cause the release of NAD, yielding satellite growth phenomenon

Satellite growth

Biochemical reaction

Catalase +ve

Oxidase +ve

Reduces nitrite to nitrate

Ferment glucose and galactose

Can't ferment sucrose, lactose and mannitol

When *Staph aureus* is streaked across plate of Blood agar with a species containing *H Influenzae*

Antigenic Properties

- Contains 3 Major surface antigens

1 Capsular polysaccharide

2 Outer membrane proteins (OMP)

3 Lipopolysaccharides (LPS)

Virulence factor of H. influnzae

- Polysaccharid capsule
- Fimbriae
- LPS- lipid A
- All virulence strain produce Neuraminidase (bioflim) and IgA protease.
- No exotoxin

Haemophilus Influenza

Mode of Transmission:

Droplet infection and discharge from the upper respiratory tract during the infectious period.

Incubation Period

Unknown, probably short, 2-4 days.

Infectious Period

- As long as the organism is present, even in the absence of nasal discharge.
- Noninfectious within 24 to 48 hours after the start of effective antibiotics

Pathogenesis

Type b *H influenzae* colonizes the nasopharynx, and may penetrate the epithelium and capillary endothelium to cause bacteremia

Meningitis may result from direct spread via lymphatic drainage or from hematogenous spread.

Nontypable *H influenzae* colonizes the nasopharynx and, to a lesser extent, the trachea and bronchi and may infect mucosa damaged by viral disease.

Lipooligosaccharide is largely responsible for inflammation

Unencapsulated *Haemophilus influenzae*

Normal flora of upper respiratory tract

Otitis media, conjunctivitis, bronchitis

Encapsulated *Haemophilus influenzae*

Type b (Hib): most virulent, invades mucosa (Ig A protease)

Bloodstream

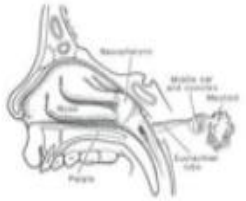
Systemic diseases

Endotoxin

Inflammation

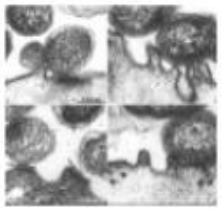
Symptoms

H. influenzae Pathogenesis

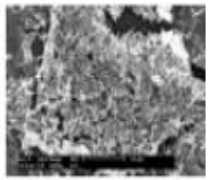


Transmission by droplet or contact

Commensal



Adherence to mucosal surface



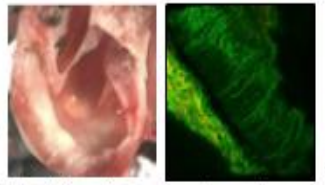
Replication/ colonization

Viral URT Infection

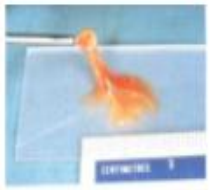
Ascension of virus-compromised Eustachian tube

Development of otitis media

Resolution of Disease



Biofilm formation in the middle ear



Species	Primary Diseases	Frequency
Haemophilus influenzae	Pneumonia, sinusitis, otitis, meningitis, epiglottitis, cellulitis, bacteremia	Common worldwide; uncommon in United States
H. aegyptius	Conjunctivitis	Uncommon
H. ducreyi	Chancroid	Uncommon in United States
H. parainfluenzae	Bacteremia, endocarditis, opportunistic infections	Rare

Clinical Presentation

Pneumonia

Septic Arthritis

Epiglottitis

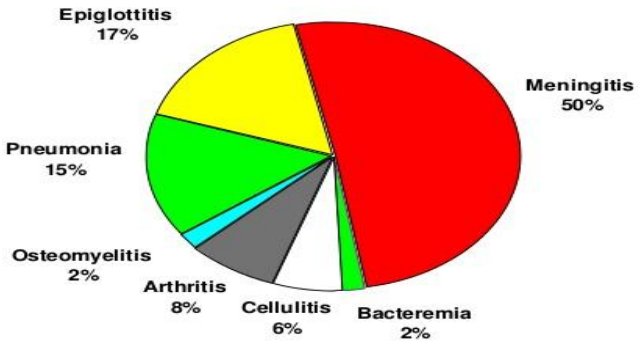
Meningitis

Invasion infection

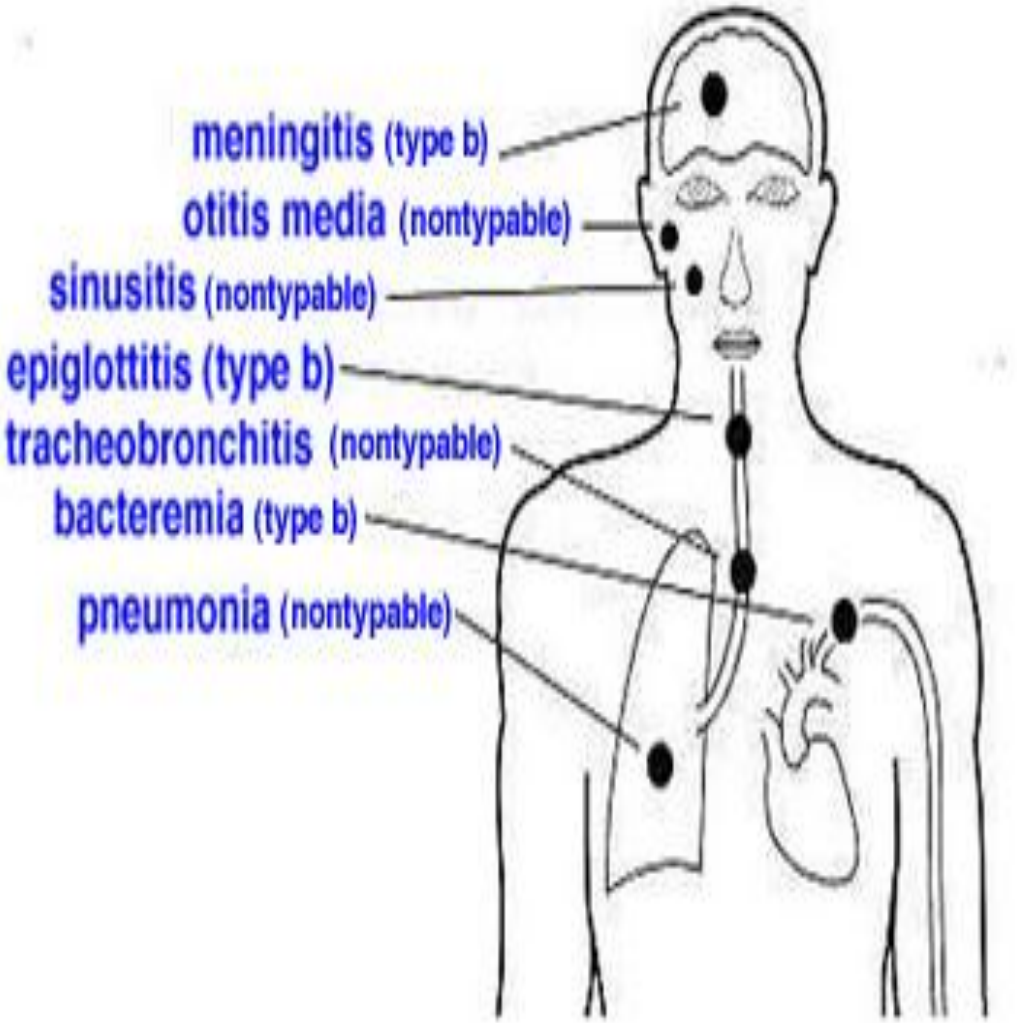
Secondary infection

- Respiratory tract infections
- Otitis media
- Sinusitis
- Chronic Bronchitis

Haemophilus influenzae type b Clinical Features*

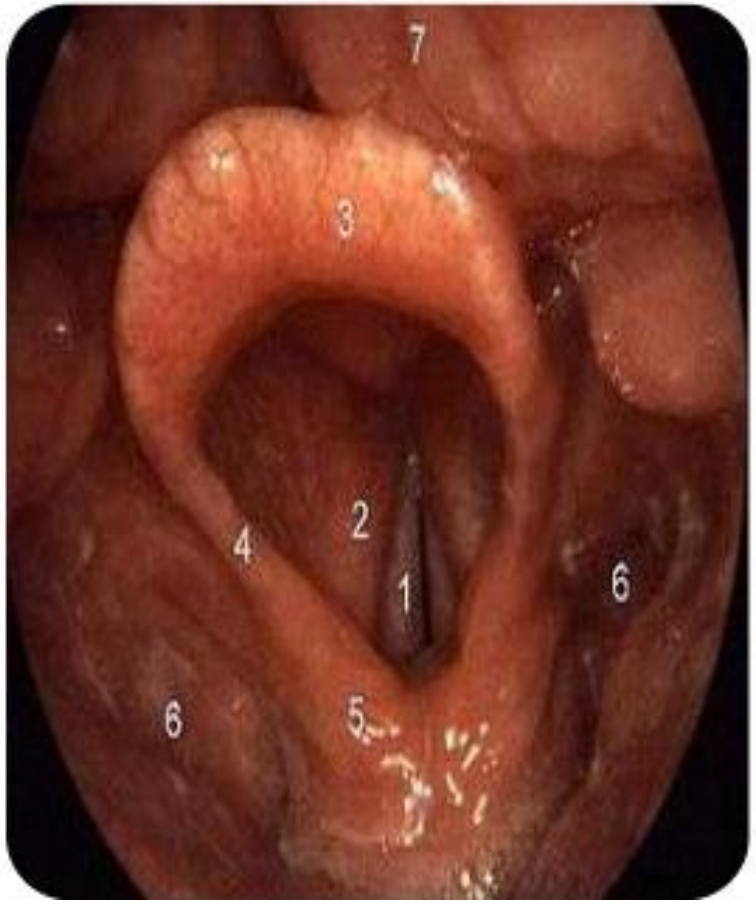


Haemophilus influenzae infections



Laryngo epiglottitis

- Causes Epiglottis
- Obstructive Laryngitis
- > 2 years children are vulnerable
- Can be fatal in 2 hours



Laboratory Diagnosis

Sample -: CSF, blood, throat swab, sputum, pus, aspirates from joints, middle ears or sinuses etc

Direct examination

Gram staining

Immunoflurescence and quelling reaction

Culturing and Isolation

Can be grown on Blood agar and Chocolate agar

Need 5 – 10 % carbon dioxide

A streak of Staphylococcus should be streaked across the plate at 370c

Opaque colonies appear shows as Satellitisim

Iridescence Demonstrates on Leviathan medium

Blood culture and CSF culture

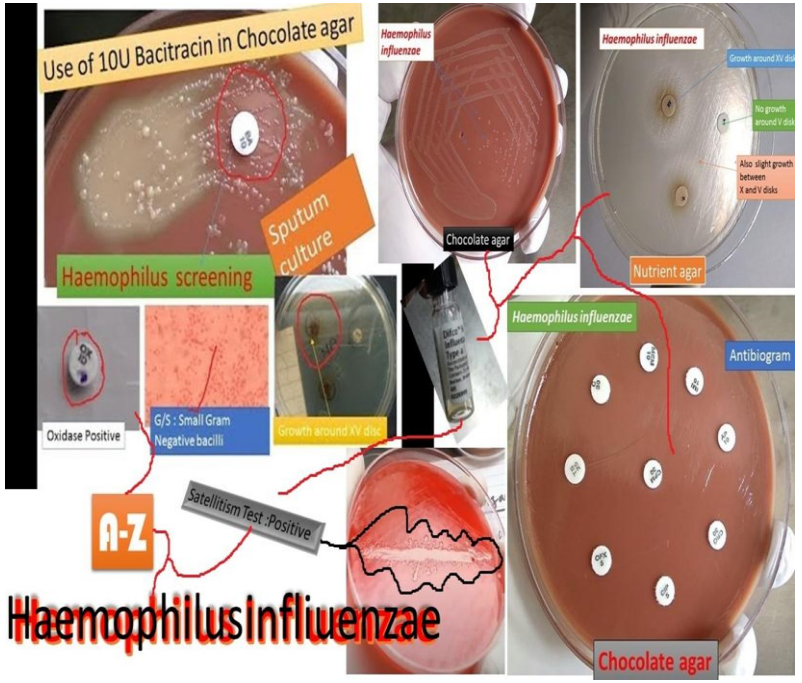
Lab diagnosis

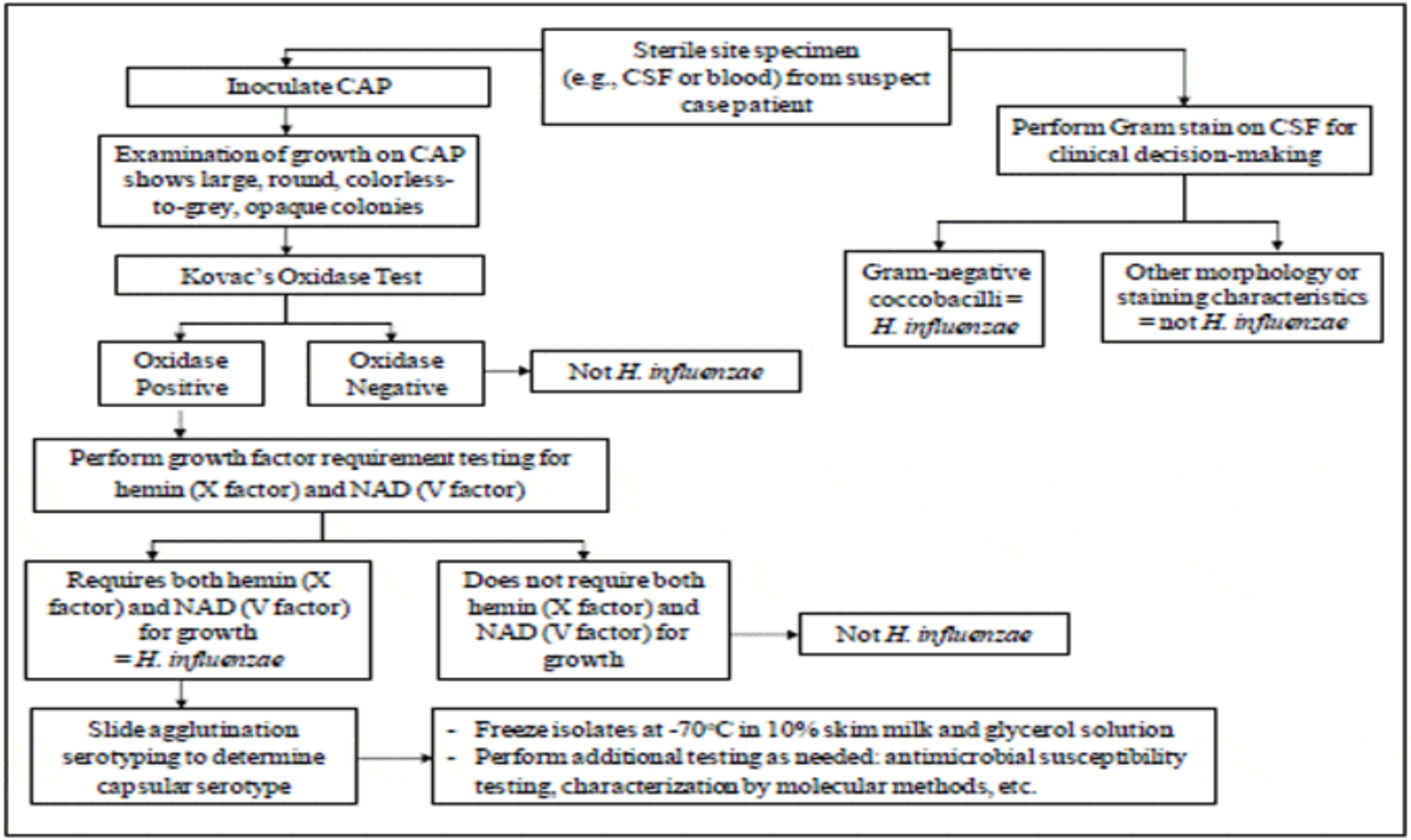
Type b Capsular antigen detection

Agglutination of latex particles

Coagglutation test

Counterimmunoelectrophoresis (CIE)




Sterile site specimen (e.g., CSF or blood) from suspect case patient
Inoculate CAP
Examination of growth on CAP shows large, round, colorless-to-grey, opaque colonies
Kovac's Oxidase Test
Oxidase Positive
Oxidase Negative
Not H. influenzae
Perform growth factor requirement testing for hemin (X factor) and NAD (V factor)
Requires both hemin (X factor) and NAD (V factor) for growth = H. influenzae
Does not require both hemin (X factor) and NAD (V factor) for growth
Not H. influenzae
Slide agglutination serotyping to determine capsular serotype
- Freeze isolates at -70°C in 10% skim milk and glycerol solution
- Perform additional testing as needed: antimicrobial susceptibility testing, characterization by molecular methods, etc.
Perform Gram stain on CSF for clinical decision-making
Gram-negative coccobacilli = H. influenzae
Other morphology or staining characteristics = not H. influenzae

Current Vaccines

- Haemophilus B conjugate vaccine
- Wide spread use of

H influenza type b vaccine has reduced H influenza type b meningitis in children by 95%

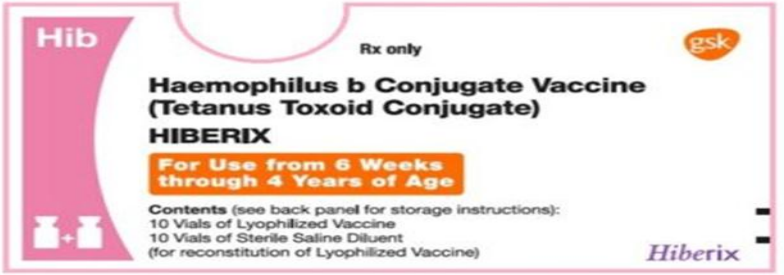
Treatment

- Cefotaxime
- Ceftazidime
- Ampicicillin, Contrimixazole
- Plasmid born resistance set in Ampicillin
- Amoxycillin with Clavulanate
- Clarithromycin
- Treatment with an effective 3rd generation cephalosporin, or chloramphenicol plus ampicillin

❑ Ampicillin-resistant strains

Hib Vaccine

Hiberix

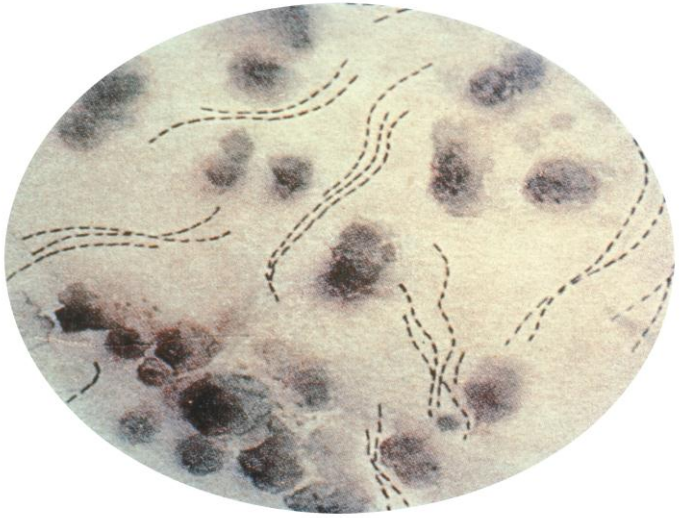


Public Health Aspect of other *Haemophilus* strains

- ***H. ducreyi***
  - **Sexually transmitted disease - chancroid**
- ***H. influenzae* biogroup *aegyptius***
  - **Brazilian Purpuric Fever**
- ***H. aegyptius***
  - **"pink eye" (purulent conjunctivitis)**
- ***H. aphrophilus***
  - **pneumonia**
  - **Infective endocarditis**

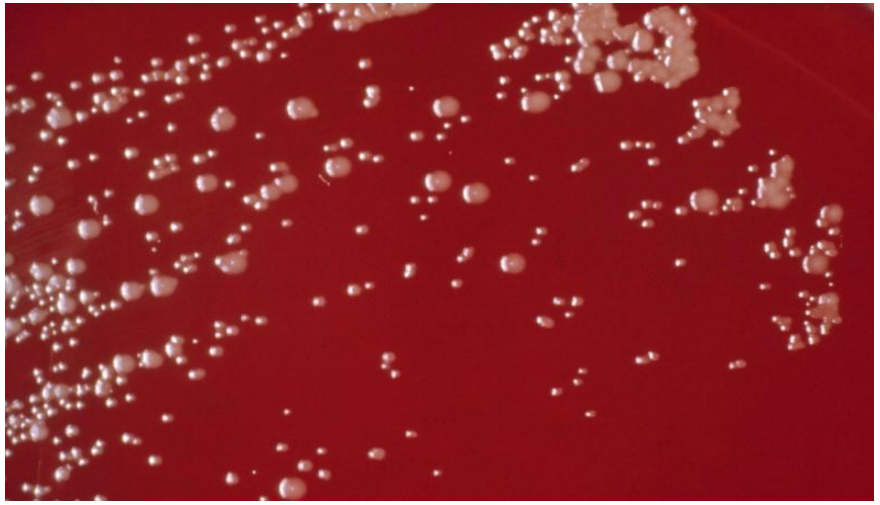
H.ducreyi

- Ducrey 1890
- Short ovoid bacilli
- 1 – 1.5 x 0.6 microns
- End to end pairing in short chains
- Gram –ve appear as Gram +ve
- Bipolar staining
- Bacilli in small groups appear as parallel chains giving school of fish appearance



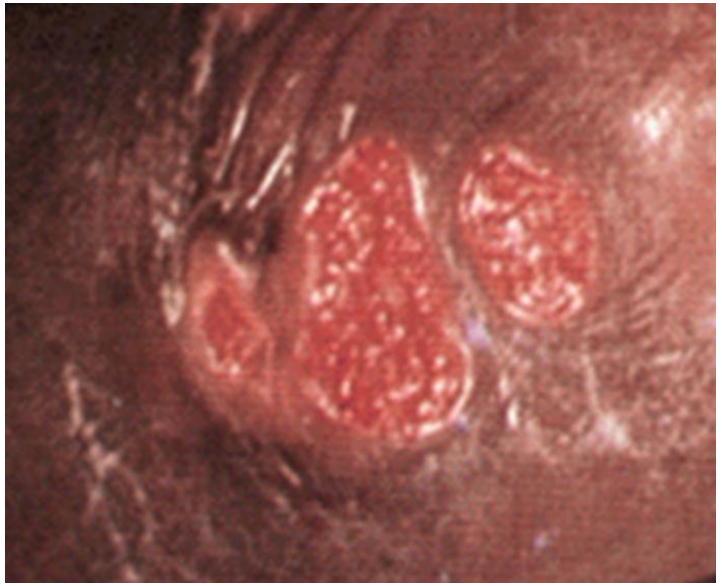
Growth and Culturing

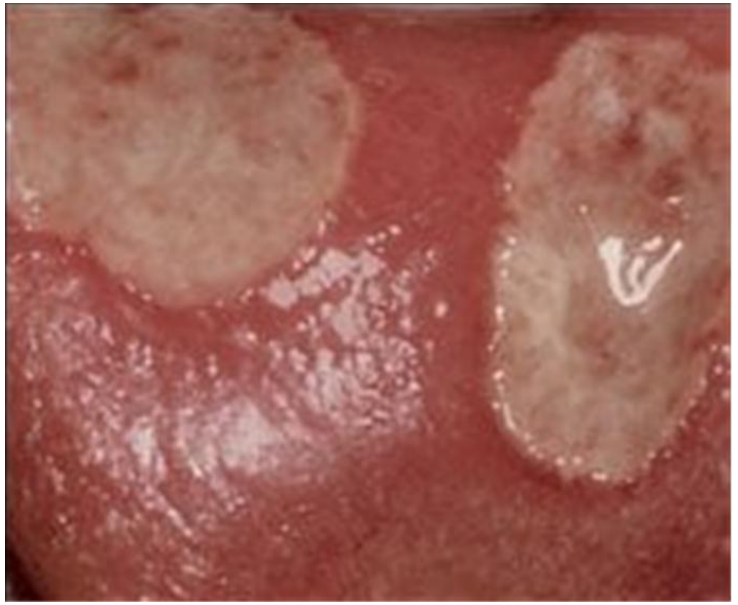
- Grows on Fresh clotted Rabbit blood
- Grows on Chorioallontoic membrane of chick embryo
- Small grey translucent colonies are produced



H.ducreyi

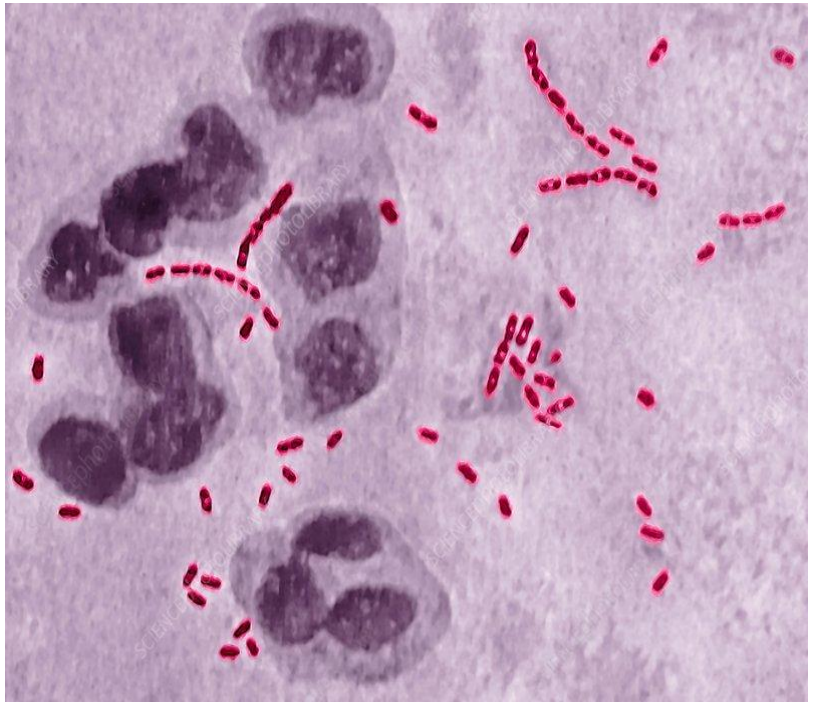
- Seen in genital regions of human
- Can be transmitted by sexually contact – STD
- In men- painful ulcer in genitals, slow healing lymphnodal enlargement, pus formation- CHANCROID- soft based ulcer
- In women – no symptom
- Infection is localized spreading to only in regional lymph nodes





Microbiological diagnostics:

In smears prepared from chancroid, characterization is based on the **microscopic** detection of small, Gram-negative bacilli with morphology.



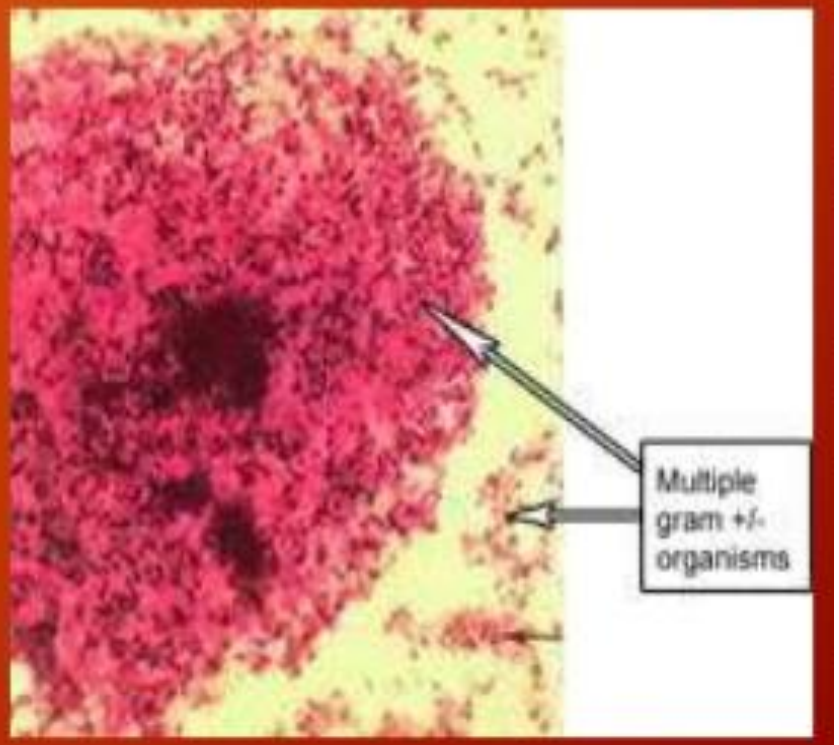
It is possible to obtain a **culture** of the causative agent and identify it by inoculating the pathological material into appropriate nutrient media.

In some cases, **PCR** is used to identify the causative agent.

Bacterial Vaginosis (Gardnerella Vaginitis)

Morphology

- Small, Gram negative, on motile
- Pleomorphic rod which shows metachromatic granules
- Presence of Clue cells



Gardnerella Vaginitis

6

- Gram-variable-staining rod, facultative anaerobic bacteria (actually has a Gram-positive cell wall, but because the cell wall is so thin it can appear either Gram-positive or Gram-negative under the microscope).
- Small (1-1.5 µm diameter) non-spore forming, non-motile coccobacilli.
- Previously classified as Haemophilus vaginalis and afterwards as Corynebacterium vaginalis.

Dr.T.V.Rao MD

Culturing

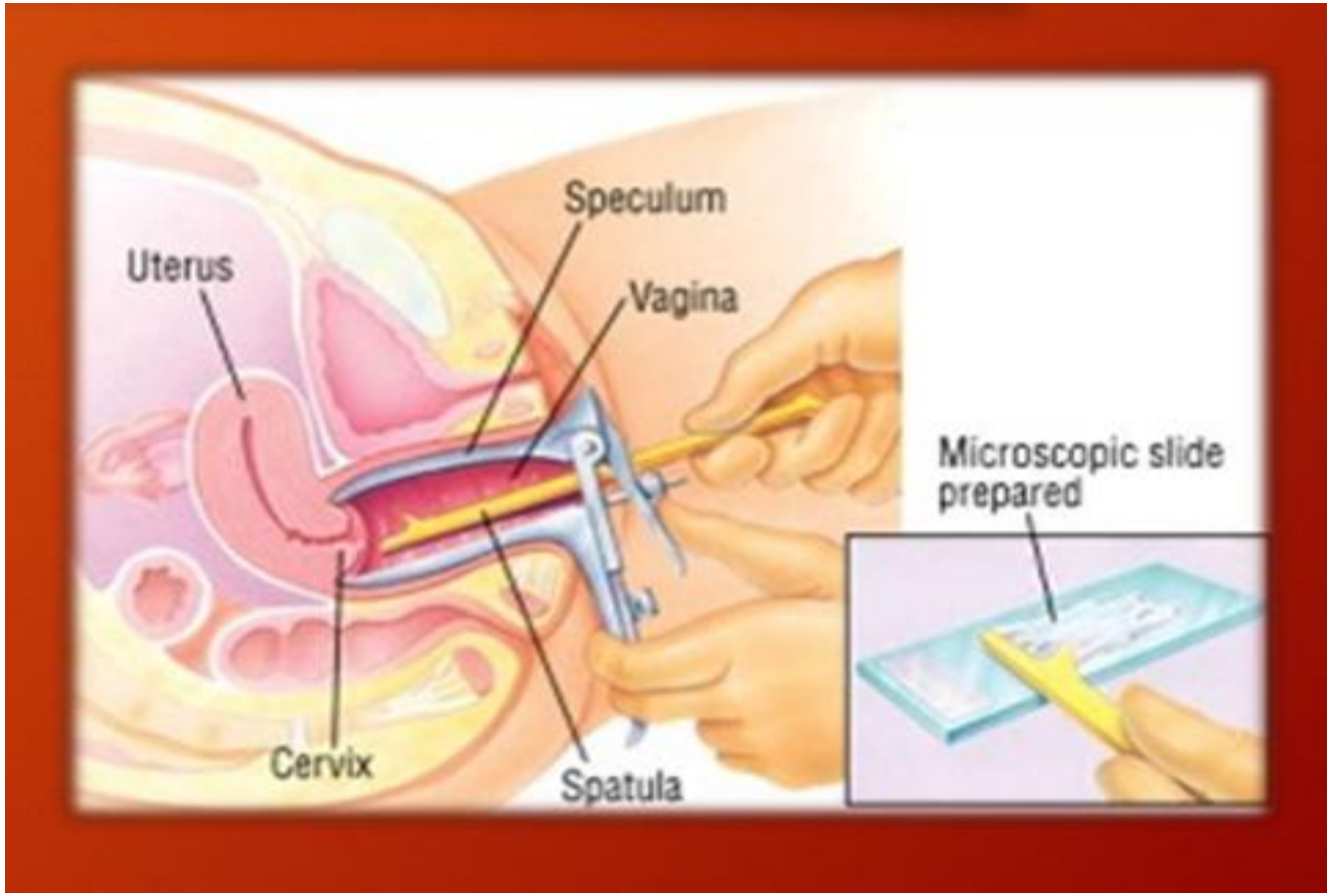
- Grows on Blood and Chocolate Agar
- Hemolytic colonies on Human and Rabbit blood agar,
- Catalase -
- Oxidase -

Symptoms

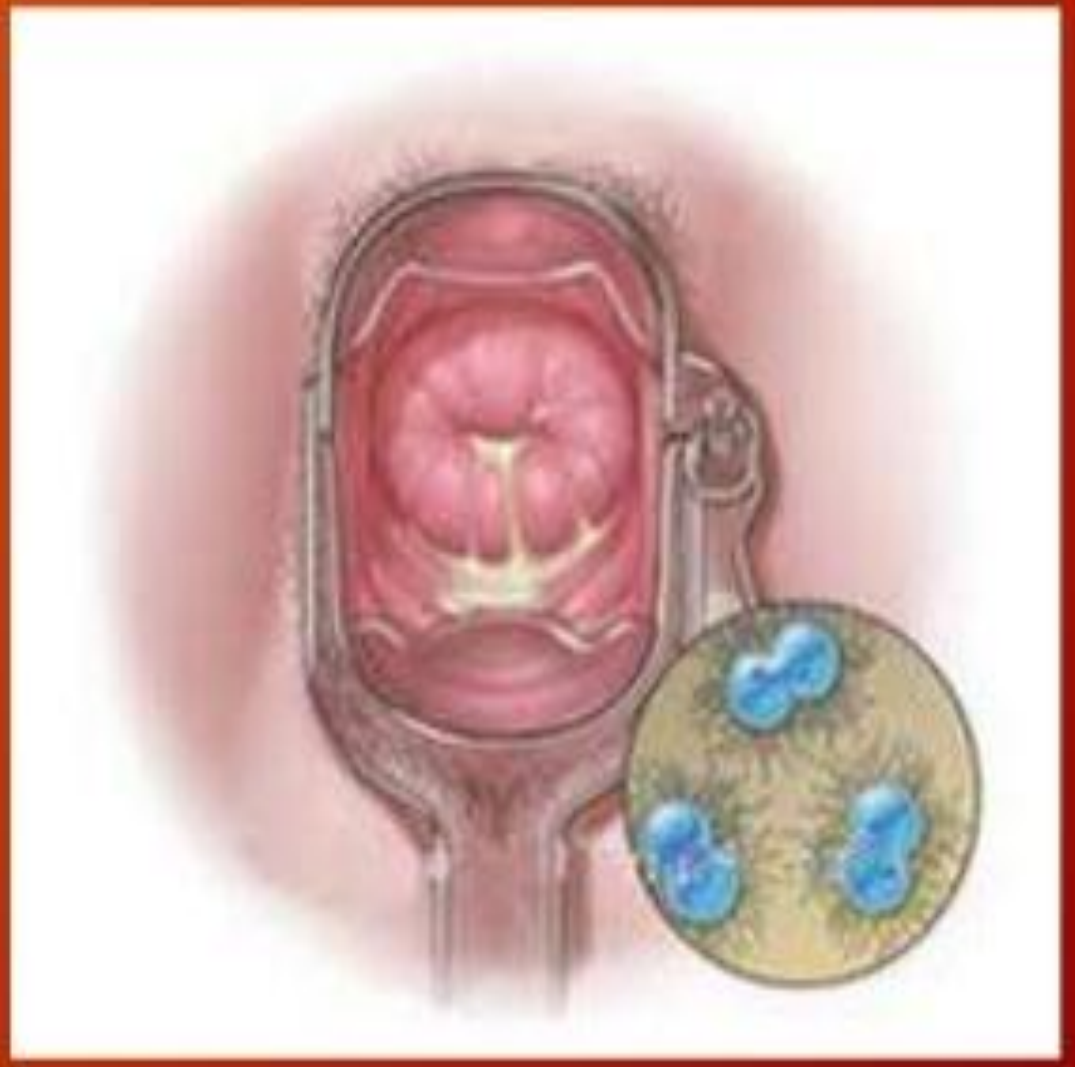
- Up to 50% of women diagnosed with bacterial vaginosis do not have symptoms. In others, it causes an unpleasant "fishy" vaginal odor and a yellow or white vaginal discharge. For some women, these symptoms are especially bothersome during or after intercourse.

Diagnosis

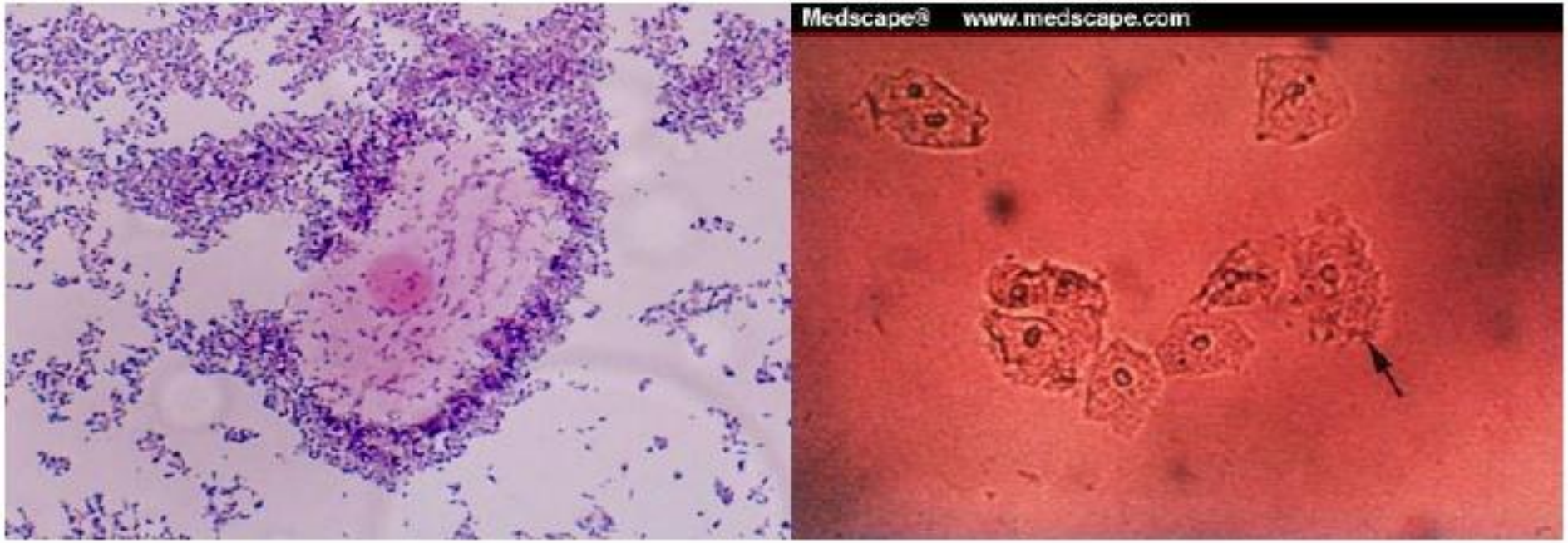
- 1 White, thin, coating on your vaginal walls during the pelvic exam



- 2 pH test of vaginal discharge that shows low acidity (pH greater than 4.5)
- 3 Fishy odor when a sample of vaginal discharge is combined with a drop of potassium hydroxide on a glass slide (the "whiff test")

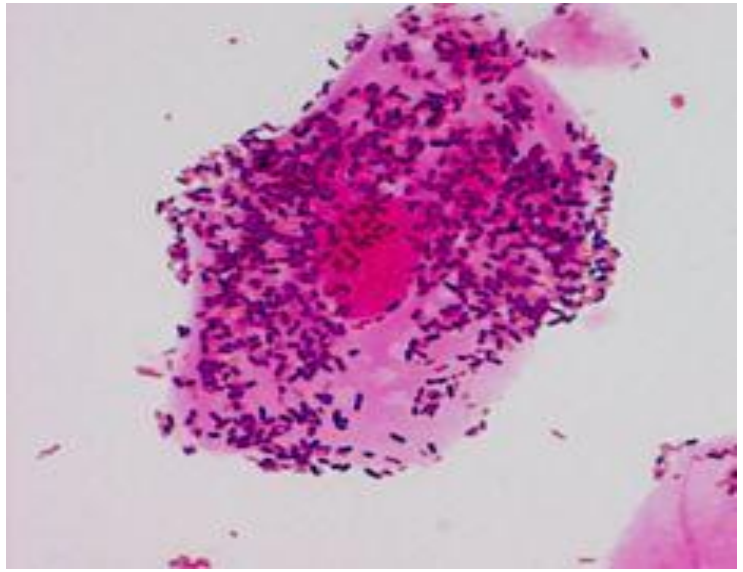


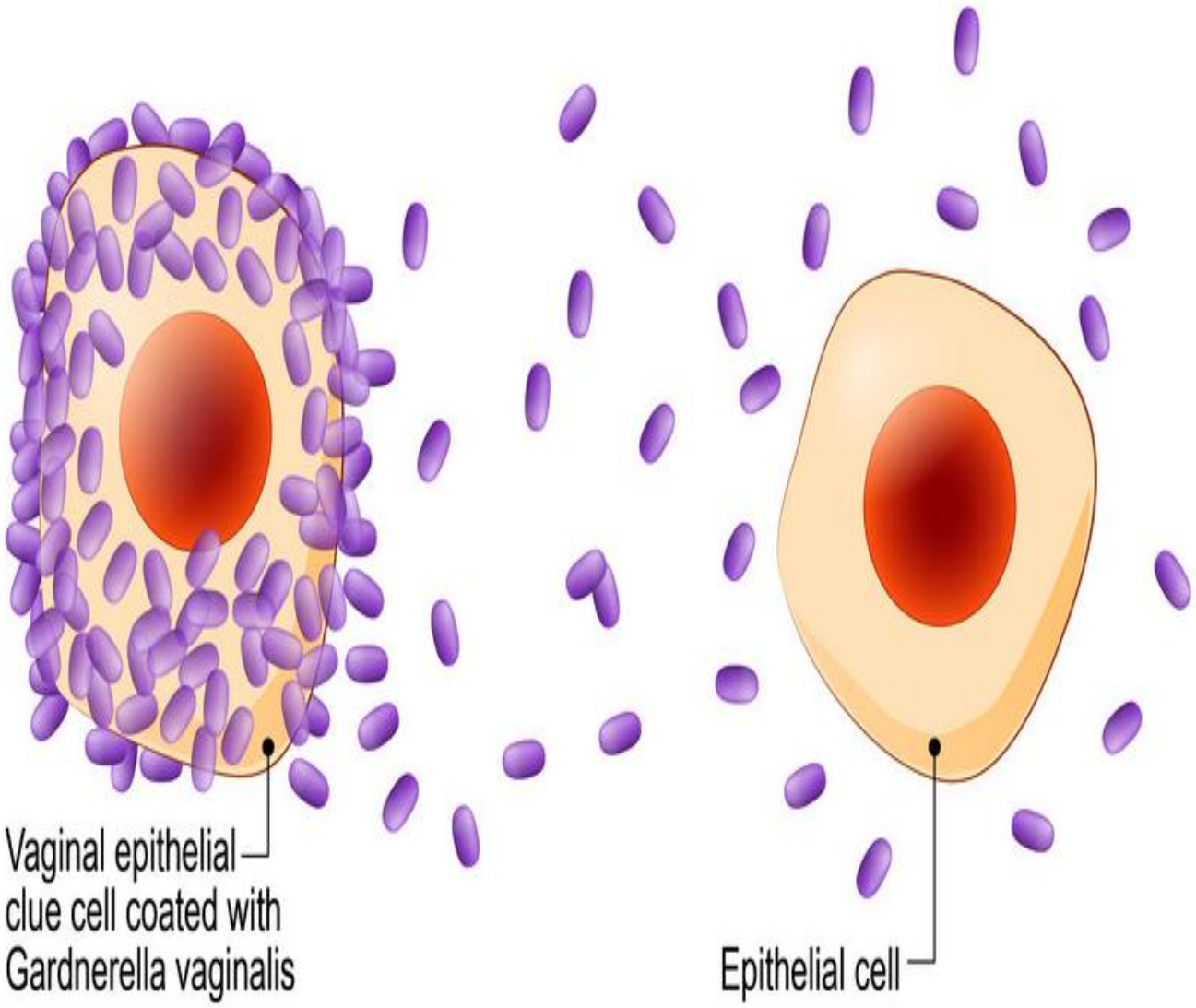
Clue cell



Clue cell on Gram stain and saline wet mount of vaginal discharge (on >20% cells)
Bacteria adhered to epithelial cells; most reliable single indicator

4 Clue cells (vaginal skin cells that are coated with bacteria) visible on microscopic exam of vaginal fluid





Treatment

- Studies show that a seven-day treatment with oral metronidazole or a five-day treatment with metronidazole vaginal gel is equally effective in non-pregnant women. Clindamycin vaginal cream is slightly less effective than either type of metronidazole.

Legionella - Taxonomy

(Domain): Bacteria

(Kingdom): Pseudomonadota

(Class): Gammaproteobacteria

(Order): Legionellales

(Family): Legionellaceae

(Genus): Legionella

(Species***):*** ***L.pneumophila,*** *L.micdadei*

Legionella pneumophila

Historical Background and Epidemiology

Historical Background

The name legionella originates from a widely publicized outbreak of pneumonia in persons attending an American Legion convention in Philadelphia in 1976.

In a hotel on the occasion of a United States army veterans' meeting (Fraser et al., 1977).

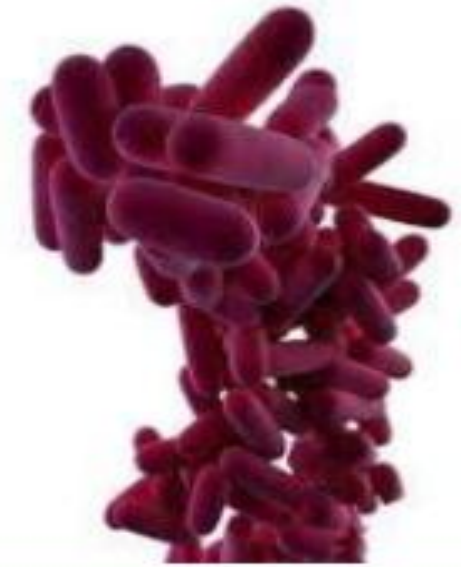
Bacterial Characteristics

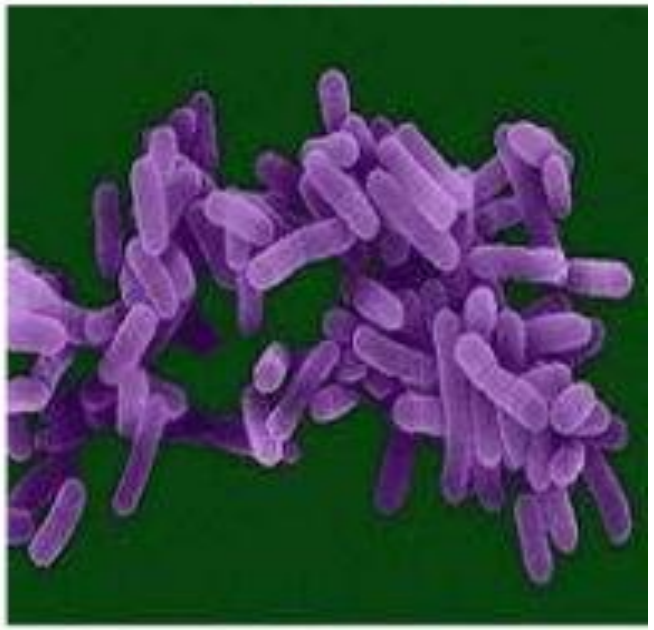
Legionellaceae are facultative intracellular parasites that cause primarily respiratory tract infections.

Legionella are :

- gram–negative
- slender rods
- unencapsulated
- fastidious,
- Aerobic
- catalase-positive
- Most produce gelatinase and ß-lactamase
- 0.5–1 m wide and 2–50 m long.
- poorly stained by Gram's stain

This has been attributed to the presence of the branched chain fatty acids that are a major component of the cell walls.





- Motile by means of one or more *polar* or *subpolar flagella*
- grown on complex media such as *buffered charcoal-yeast extract (BCYE) agar* with ;

  ketoglutarate,

  pH of 6.9,

  temperature 35 °C, and

  90% humidity.
- Legionellae grow slowly;

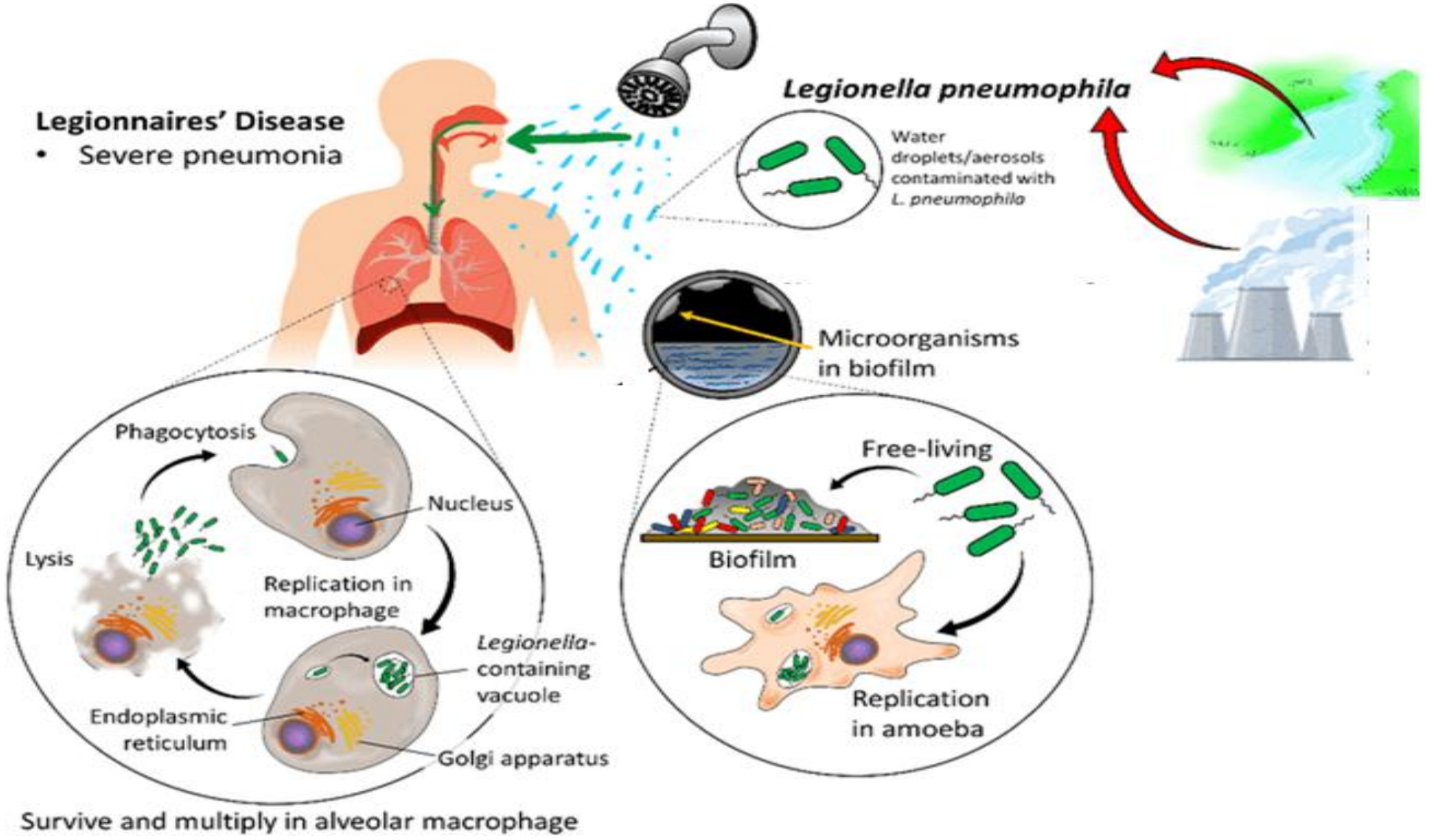
  3 days of incubation with BCYE & ≥2 weeks in blood cultures
- Colonies are round or flat, colorless to iridescent pink or blue

Virulence factors

The Legionellae make:

- proteases,
- phosphatase,
- lipase,
- DNase, and
- Rnase
- A major secretory protein, a ***metalloprotease,*** has hemolytic and cytotoxic activity; however, this protein **has not been shown** to be a required virulence factor.

Source of infection and mode of transmission



Pathogenesis

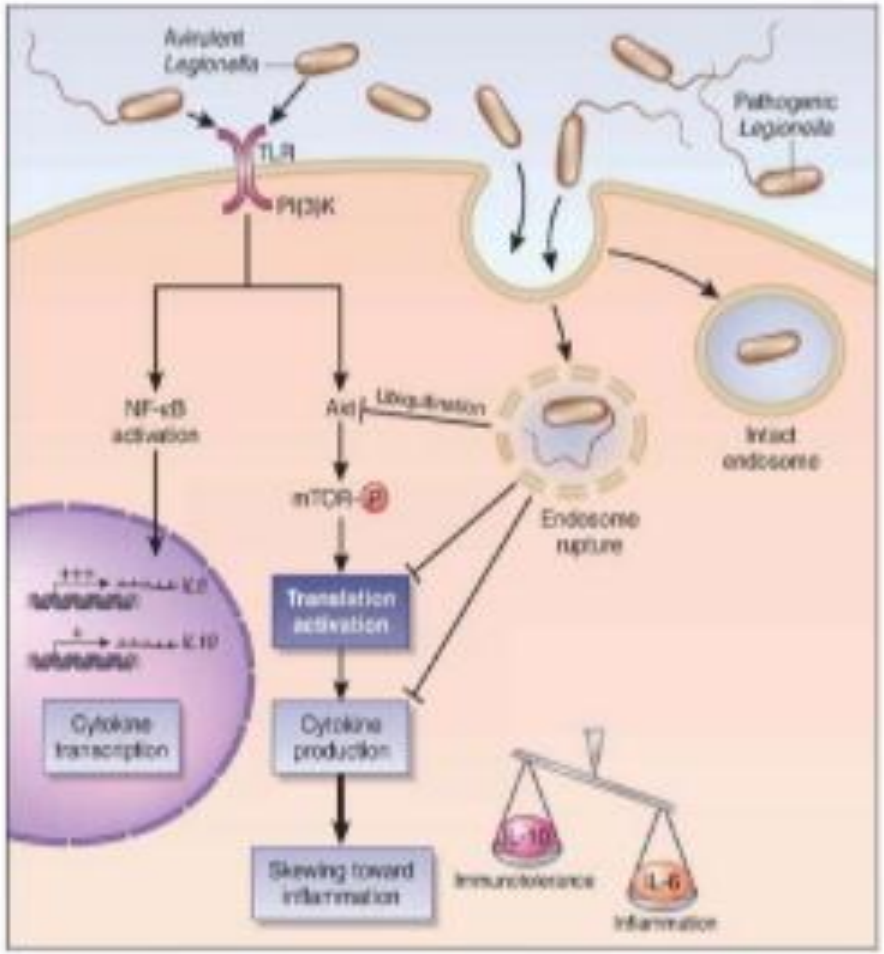
Legionellae are intracellular pathogens of ***macrophages***, by which they are phagocytosed in a process involving ;

1. Both virulent and non-virulent strains are *phagocytosed*
2. Virulent strains can multiply inside the phagocytes and are able to ***inhibit the fusion of phagosomes with lysosomes***

- ❑ non-virulent strains do not multiply

3. The bacteria multiply within the vacuoles until they are numerous,
4. The cells are destroyed, the bacteria are released, and infection of other macrophages then occurs.

(transferrin-iron) is essential for the process of *intracellular growth* of the bacteria, but other factors important to the processes of *growth, cell destruction, and tissue damage* are not well understood.



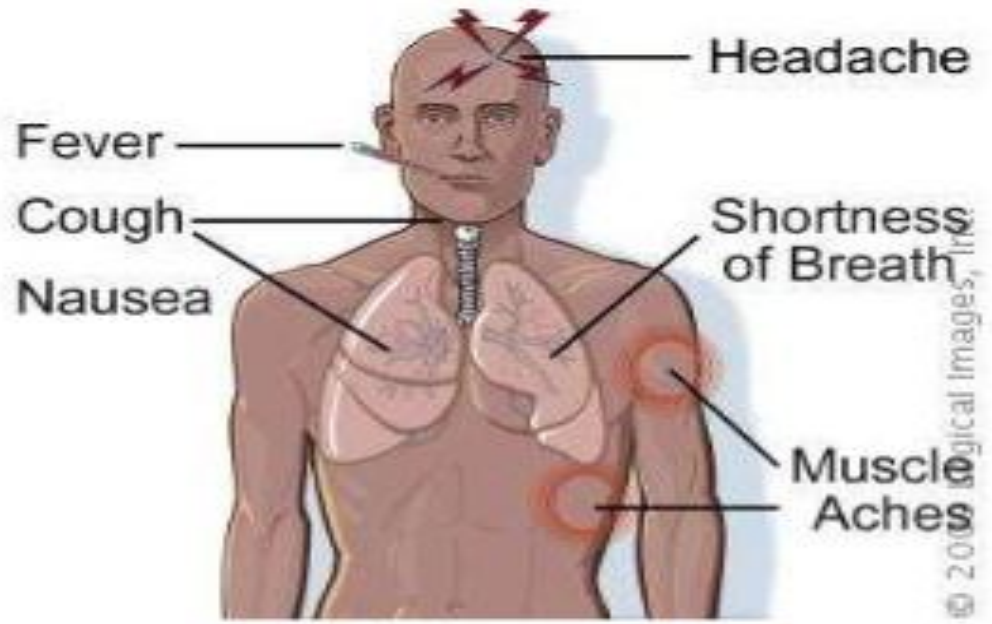
Clinical manifestations

- *L.neumophilia* causes **Legionnaires' disease** can have symptoms like many other forms of pneumonia, so it can be hard to diagnose at first.

Signs of Legionnaires' disease can include:

- Cough
- Shortness of breath
- High fever
- Muscle aches
- Headaches

These symptoms usually begin 2 to 14 days after being exposed to the bacteria.



Potanic fever

- *L .pneumophila* also produces a disease called **"Pontiac fever,"** after the clinical syndrome that occurred in an outbreak in Michigan.

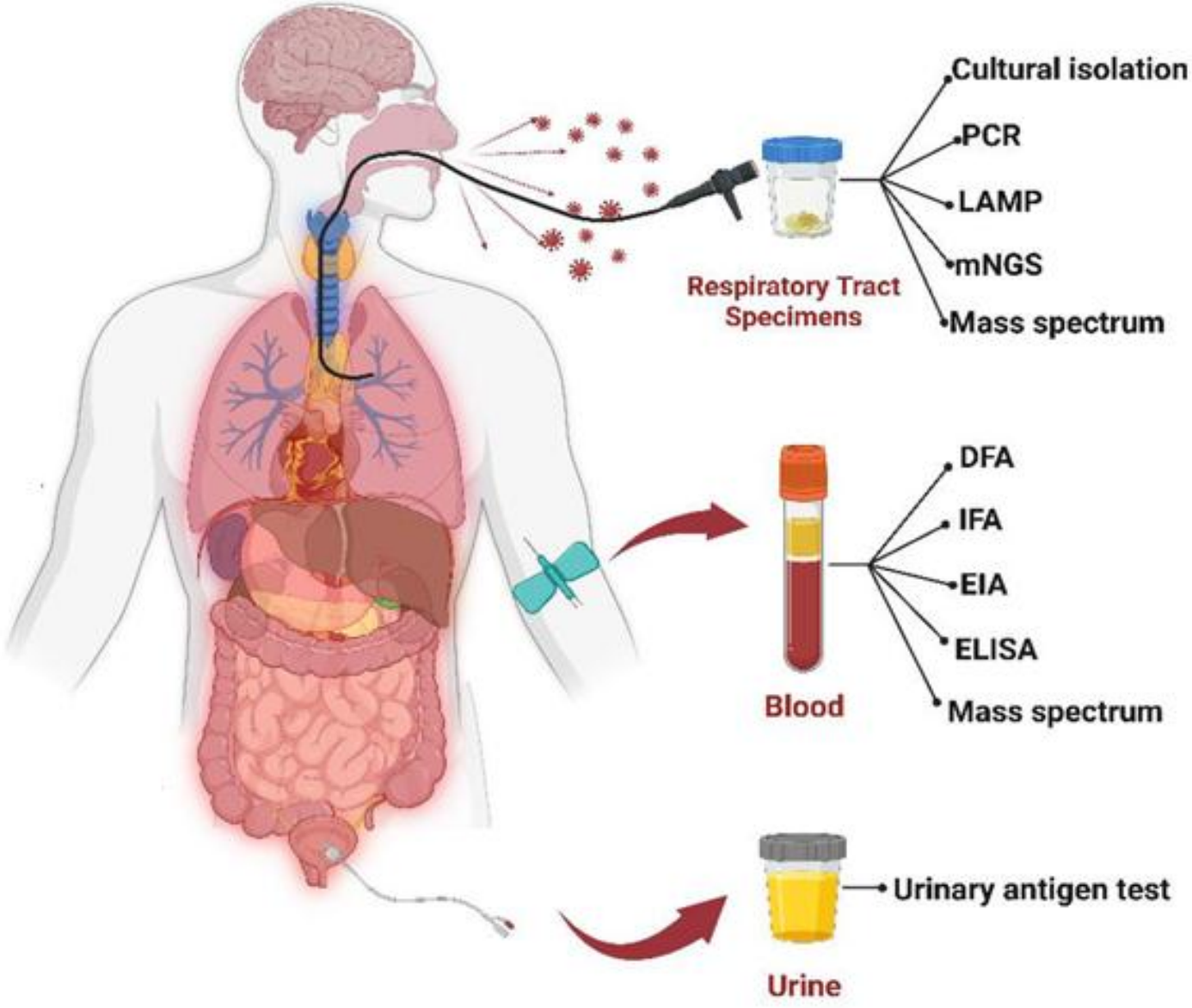
characteristics

- ❑ fever and chills,
- ❑ myalgia,
- ❑ malaise, and
- ❑ headache ,that develop over *6–12 hours*. Dizziness, photophobia, neck stiffness, and confusion also occur.

- The **symptoms of Pontiac fever** are similar to those of Legionnaires' disease and usually last for 2 to 5 days. Pontiac fever is ***different from Legionnaires' disease*** because the patient **does not** have pneumonia.
- Symptoms go away on their own without treatment.

Laboratory Diagnosis

- Specimens
  - Sputum
  - Bronchial aspirate
  - Lung biopsy
  - Florescent methods
- Serology ELISA



Specimens

The organisms can be recovered from:

- bronchial washings
- pleural fluid
- lung biopsy specimens or
- blood

❖ Isolation of legionella from sputum is more difficult because of the predominance of bacteria of the normal flora. Legionella is rarely recovered from other anatomic sites.

Smears

- Legionellae are ***not*** demonstrable in Gram-stain
- *Direct fluorescent antibody tests*, but the test has low sensitivity compared with culture
- *Silver stains* are sometimes used on tissue specimens.

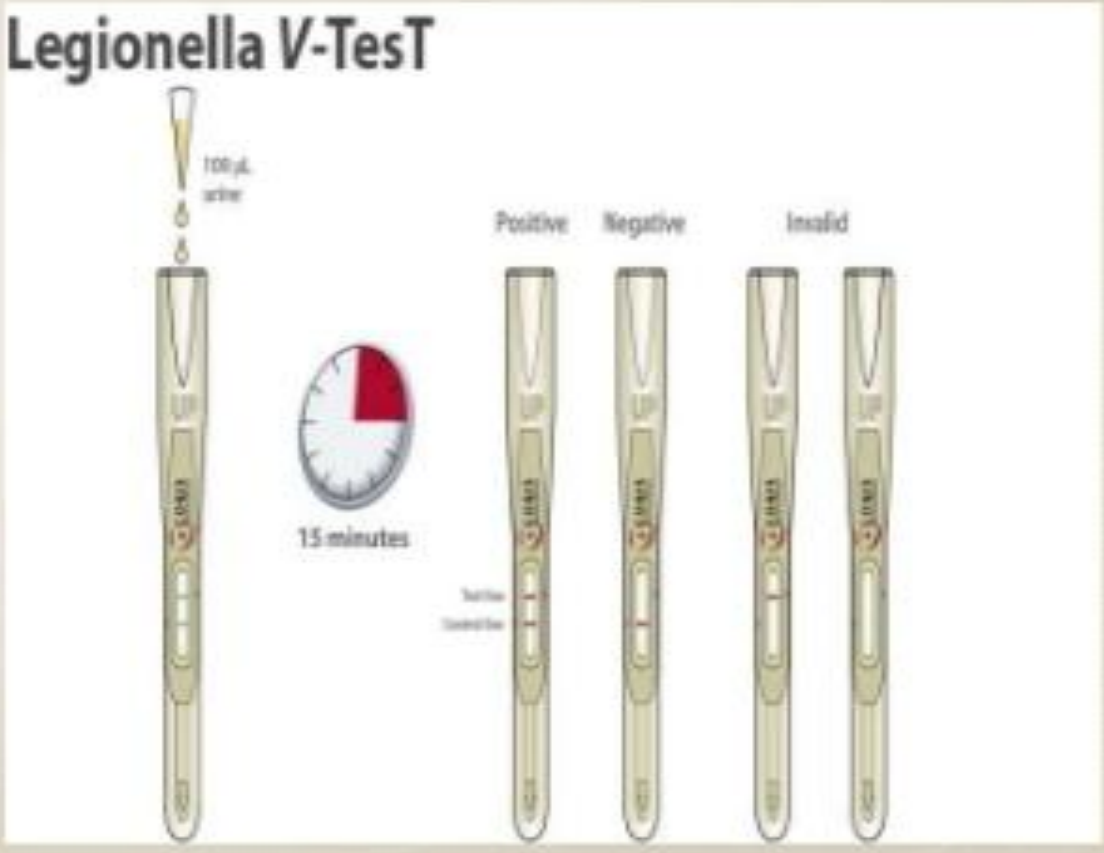
Culture

Specimens are cultured on **BCYE agar** & can be rapidly identified by immunofluorescence staining.

BCYE agar containing antibiotics can be used

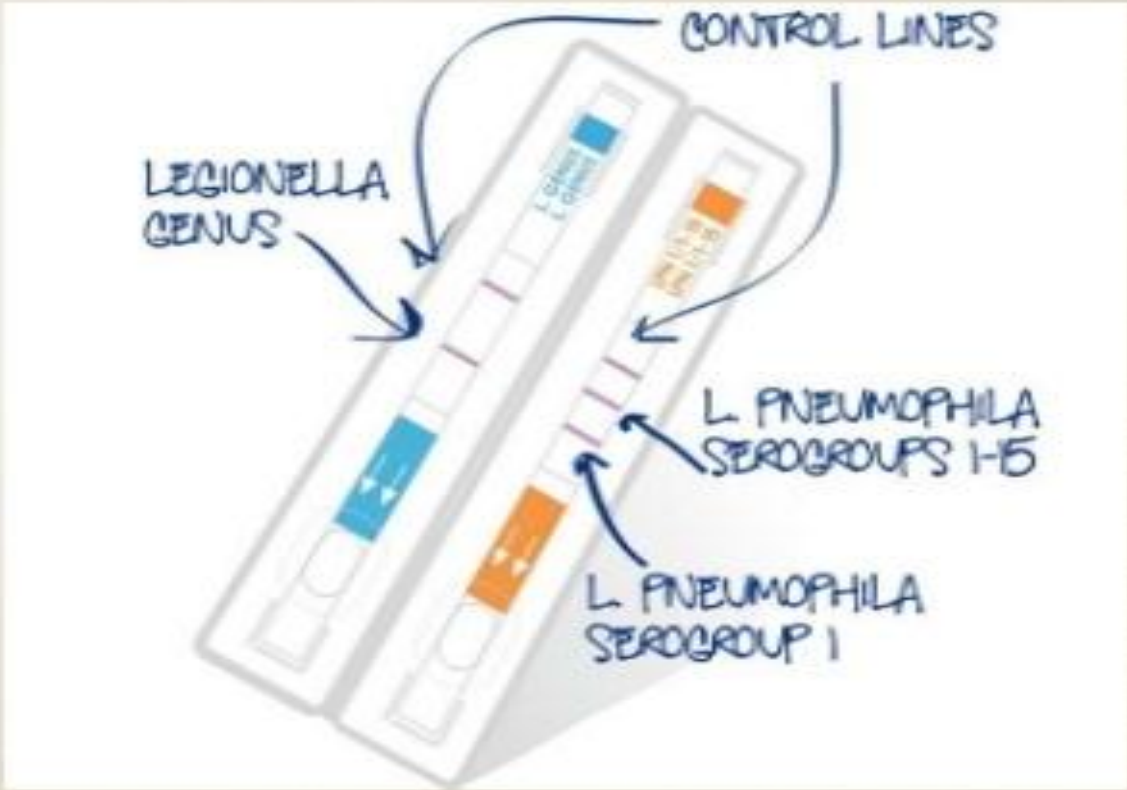
Urine Antigen Test

- The most commonly used laboratory test for diagnosis is the urinary antigen test, which detects a part of the Legionella bacteria in urine (pee). If the patient has pneumonia and the test is positive, then the patient is considered to have Legionnaires' disease.



Blood Specimens Testing the Serum

* Paired sera (blood specimens) that show a four-fold increase in antibody levels when drawn shortly after illness and several weeks following recovery, can also be used to confirm the diagnosis.

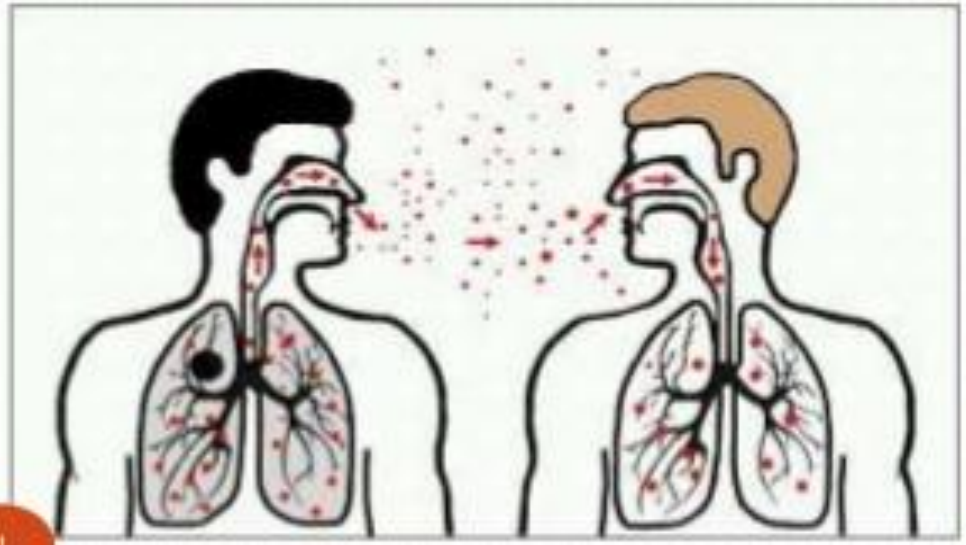


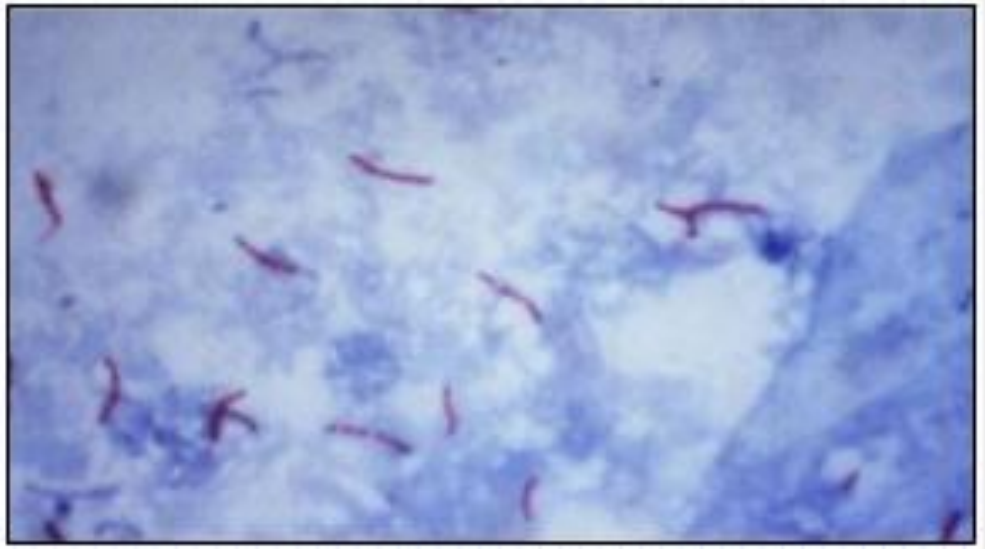
Treatment

* Macrolides
* Ciprofloxacin
* Tetracycline's
* Rifampicin

PULMONARY TUBERCULOSIS

MYCOBACTERIUM TUBERCULOSIS





INTRODUCTION

- Tuberculosis is a worldwide public health problem

Classification of Mycobacteria

1. **Tubercle bacilli**
   a) **Human – MTB**
   b) Bovine – *M. bovis*
   c) Murine – *M. microti*
   d) Avian – *M. avium*
   e) Cold blooded – *M. marinum*

   MTB Complex (*M. africanum* also included)

2. **Lepra bacilli**
   a) Human – *M. leprae*
   b) Rat – *M. leprae murium*
3. **Mycobacteria causing skin ulcers**
   a) *M. ulcerans*
   b) *M. belnei*
4. **Atypical Mycobacteria (Runyon Groups)**
   a) Photochromogens
   b) Scotochromogens
   c) Nonphotochromogens
   d) Rapid growers
5. **Johne's bacillus**

   M. paratuberculosis
6. **Saprophytic mycobacteria**
   a) *M. butyricum*
   b) *M. phlei*
   c) *M. stercoralis*
   d) *M. smegmatis*
   e) Others

What are Mycobacteria?

- Obligate **aerobes** growing most successfully in tissues with a high oxygen content, such as the lungs.
- Facultative **intracellular pathogens** usually infecting **mononuclear phagocytes** (e.g. macrophages).

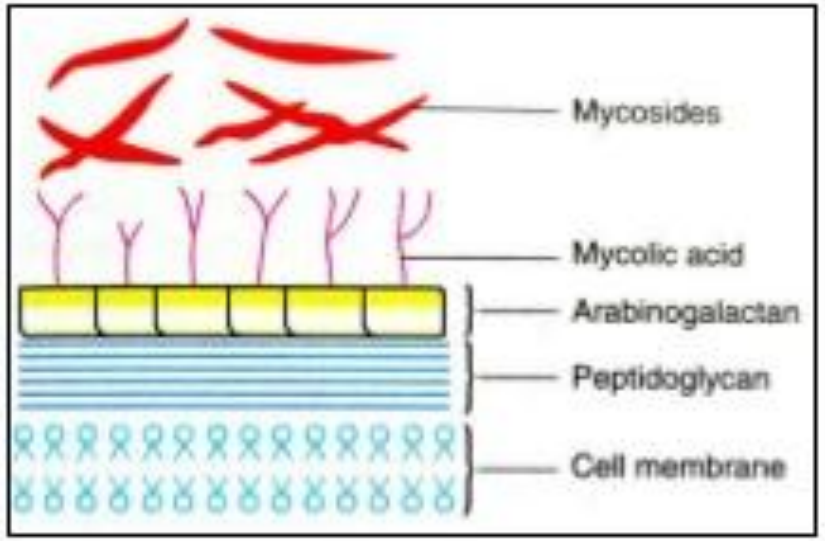
Mycobacterium differ from other routinely isolated Bacteria

- **Slow-growing** with a generation time of 14 to 15 hours (20-30 minutes for *Escherichia coli*).
- **Hydrophobic** with a high lipid content in the cell wall. As they are hydrophobic and tend to clump together, they are impermeable to the usual stains,

  e.g. **Gram's stain**

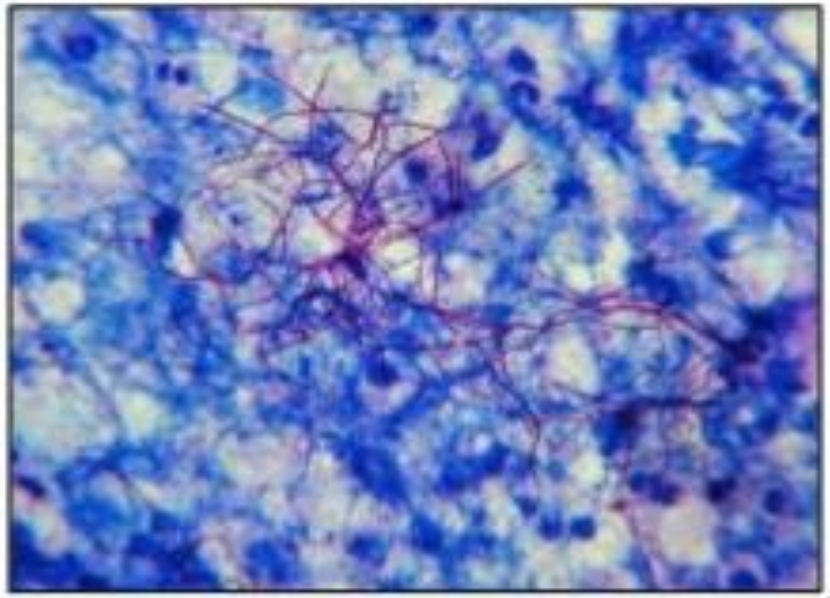
Acid fast bacilli

- Known as **"Acid-fast bacilli"** because of their lipid-rich cell walls, which are relatively impermeable to various basic dyes unless the dyes are combined with phenol.



How they are Acid fast

- Once stained, the cells resist decolourization with acidified organic solvents and are therefore called "acid-fast". (Other bacteria which also contain mycolic acids, such as *Nocardia*, can also exhibit this feature.)



Mycobacterium tuberculosis

MORPHOLOGY:-

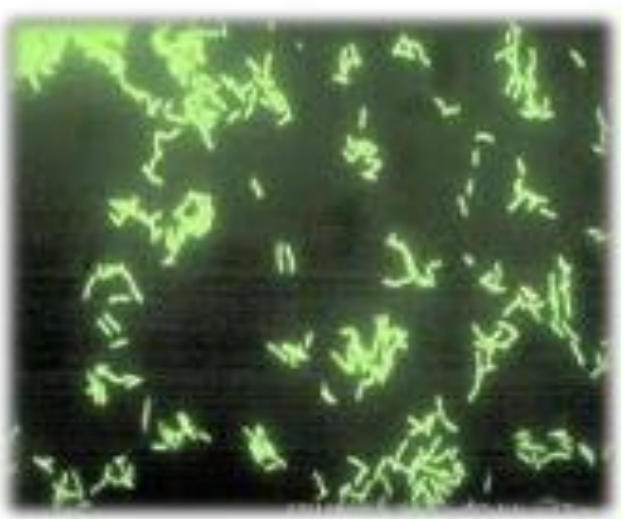
- Slender, straight or slightly curved bacilli with rounded ends, occurring singly or in pairs or in clumps.
- Non-sporing, non-capsulated and non-motile.

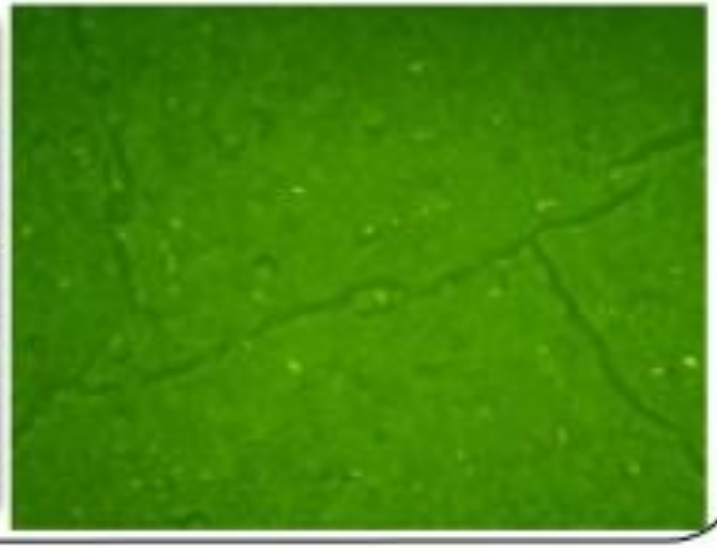
1. **Ziehl Neelsen stain** – stained by carbol fuschin; heat melts wax; resist decolourisation by 20% sulphuric acid . Resist decolourization by absolute alcohol.

   (Acid fast and alcohol fast)

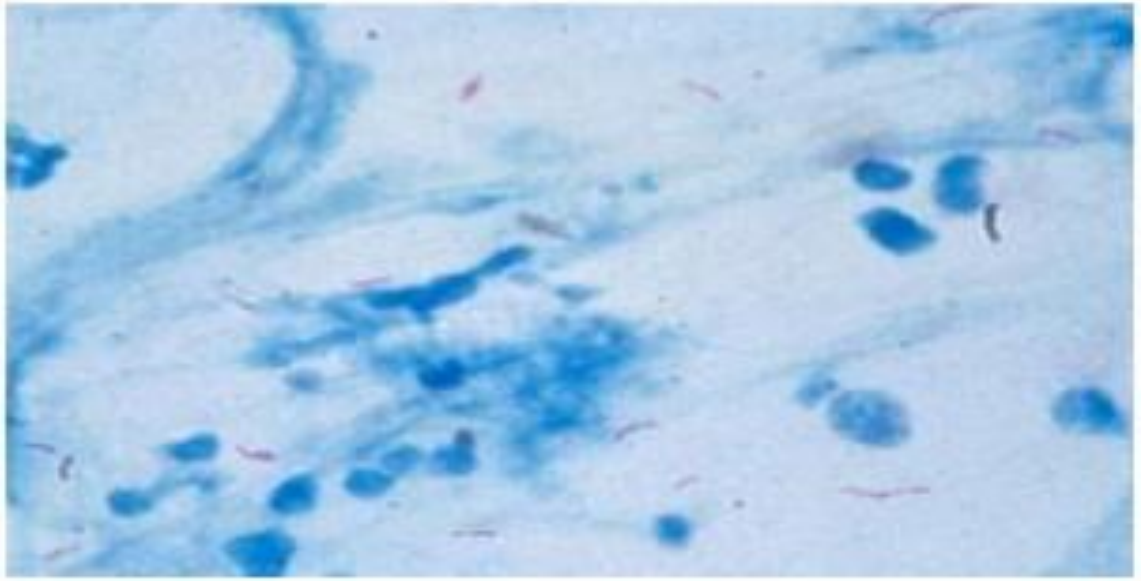
2. **Auramine rhodamine stain**

   (fluorescent stain)



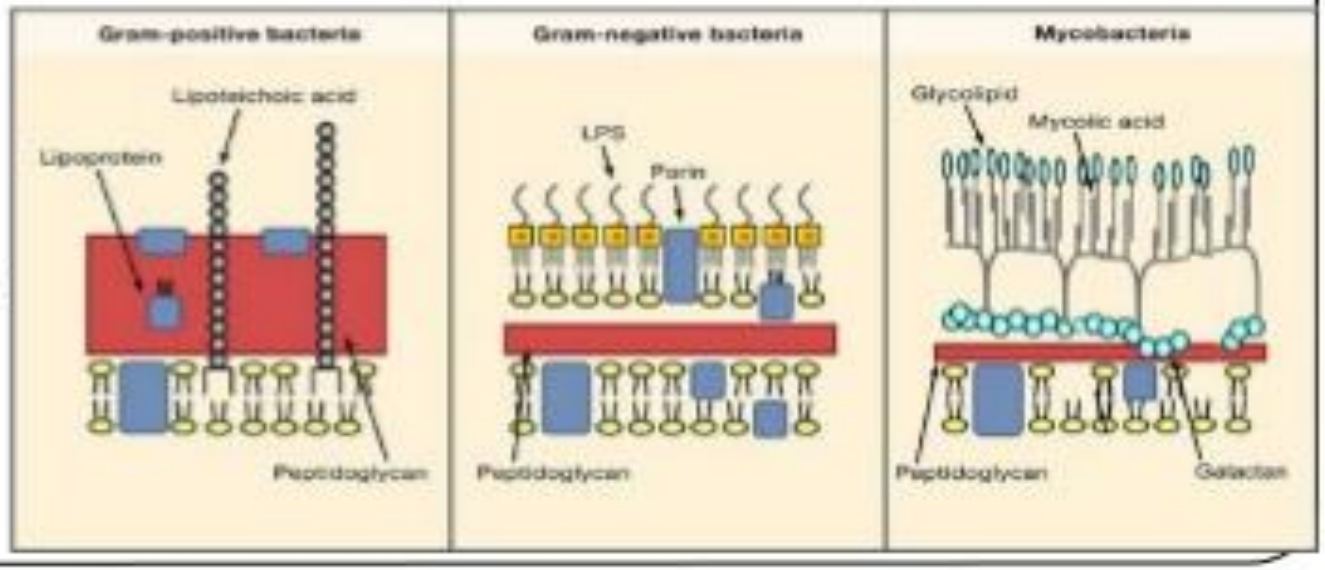


- **Acid fast bacilli**
- Straight or slightly curved.
- 1- 4 x 0.2-0.8 µm.
- Single, small clumps, pairs, long filamentous forms may be seen.
- Other (bacteria, cells – stained blue by)
- Counter stain (methylene blue)



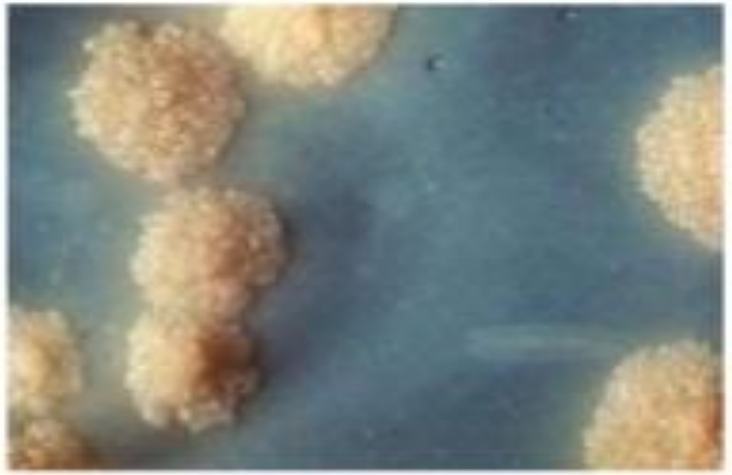
COUNTER STAINS USED:-

- Methylene blue – Blue background
- Malachite green – Green ”



CULTURAL CHARACTERS:-

- Aerobe.
- Growth stimulation by 5-10% CO_2
- Bacilli grow slowly, generation time 14- 15 hrs.
- Colonies appear in about two weeks or delayed upto 6-8 weeks.
- Optimum temp. 37°c
- Optimum pH 6.4-7.0
- Colonies - rough, tough and buff
- *M. tuberculosis* – obligate aerobe
- *M. bovis* – Microaerophilic



1. **Solid media:-**
   i. Containing egg – **Lowenstein Jensen**, Petragnin, Dorset's egg.
   ii. Containing blood – Tarshis medium.
   iii. Containing potato – Pawlowsky's medium.

- Medium most commonly used is Lowenstein Jensen medium contain:-
   i. Coagulated hen's eggs (neutralise fatty acid)
   ii. Glycerol (C source)
   iii. Mineral salt solution
   iv. Asparagines (nitrogen source)
   v. Malachite green (inhibits growth of other bacteria)

2. Liquid media:-

- Dubo's, Middlebrooke's, Prouskeur & Beck's, Sula's & Sauton's.

- Liquid media useful for – sensitivity tests, for extraction of Ag & vaccines.
  i. Growth in liquid media- pellicle at surface.
  ii. Dubo's medium with tween 80 – diffuse growth

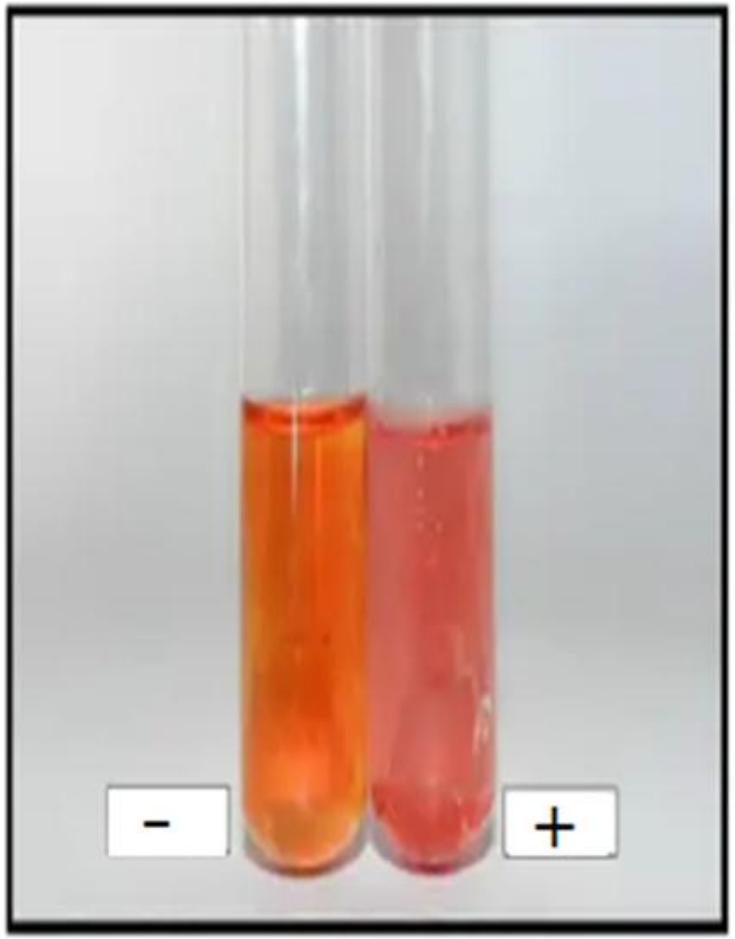
- **Virulent strain – Serpentine cords**
- **Avirulent strain – Dispersed growth.**

- Tubercle bacilli also grow in chick embryo & tissue culture.

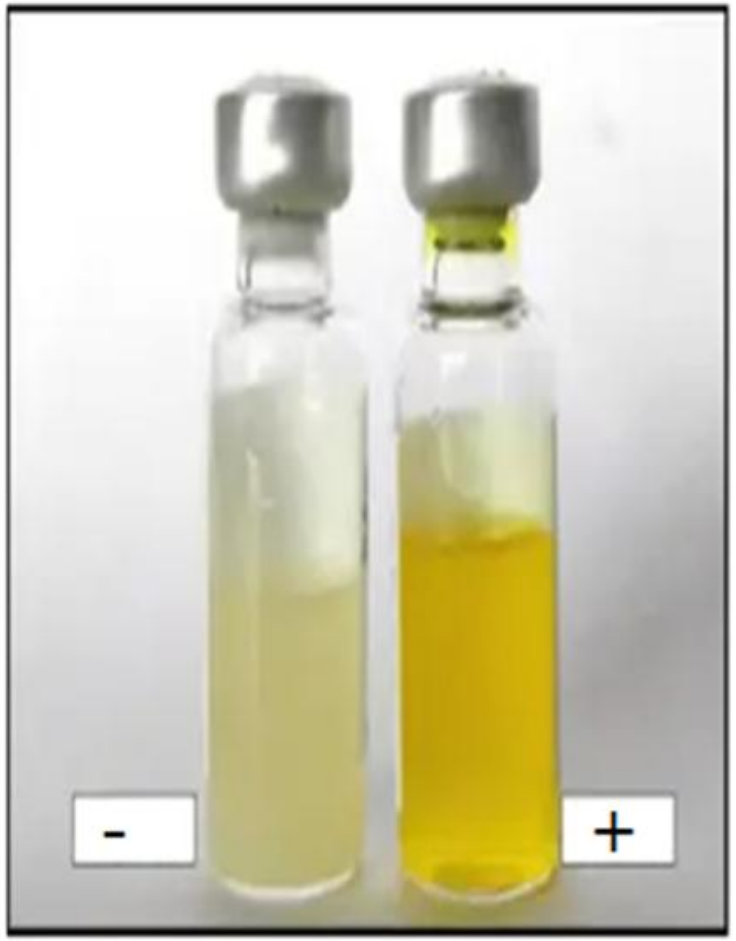
Organism	Pathogenicity	Frequency in United States
***MYCOBACTERIUM TUBERCULOSIS* COMPLEX**		
M. tuberculosis	Strictly pathogenic	Common
M. leprae	Strictly pathogenic	Uncommon
M. africanum	Strictly pathogenic	Rare
M. bovis	Strictly pathogenic	Rare
M. bovis BCG (bacillus Calmette-Guérin strain)	Sometimes pathogenic	Rare
SLOW-GROWING NONTUBERCULOUS MYCOBACTERIA		
M. avium complex	Usually pathogenic	Common
M. kansasii	Usually pathogenic	Common
M. marinum	Usually pathogenic	Uncommon
M. simiae	Usually pathogenic	Uncommon
M. szulgai	Usually pathogenic	Uncommon
M. genavense	Usually pathogenic	Uncommon
M. haemophilum	Usually pathogenic	Uncommon
M. malmoense	Usually pathogenic	Uncommon
M. ulcerans	Usually pathogenic	Uncommon
M. scrofulaceum	Sometimes pathogenic	Uncommon
M. xenopi	Sometimes pathogenic	Uncommon
RAPIDLY GROWING NONTUBERCULOUS MYCOBACTERIA		
M. abscessus	Sometimes pathogenic	Common
M. chelonae	Sometimes pathogenic	Common
M. fortuitum	Sometimes pathogenic	Common
M. mucogenicum	Sometimes pathogenic	Common
NOCARDIA		
N. cyriacigeorgica	Usually pathogenic	Common
N. farcinica	Usually pathogenic	Common
N. abscessus	Usually pathogenic	Uncommon
N. beijingensis	Usually pathogenic	Uncommon
N. brasiliensis	Usually pathogenic	Uncommon
N. nova	Usually pathogenic	Uncommon
N. otitidiscaviarum	Usually pathogenic	Uncommon
Nocardia spp.	Sometimes pathogenic	Rare
Rhodococcus equi	Usually pathogenic	Common
Gordonia spp.	Sometimes pathogenic	Rare
Tsukamurella spp.	Sometimes pathogenic	Rare

Classification of Selected Acid-Fast Bacteria Pathogenic for Humans

	M. tuberculosis	M. bovis
Morphology	Long, slender and usually curved	Short, stout and straight
Staining	Barred or beaded appearance	Uniform staining
Growth on LJ medium	Eugonic	Dysgonic
Presence of glycerol in medium	Enhances the growth	Inhibits the growth
Colony	Dry, rough, tough, raised & wrinkled, difficult to emulsify	Moist, smooth, flat, white and friable
Biochemical reactions		
Niacin test	+	-
Nitrate reduction	-	-
Animal pathogenicity		
In guinea pig	+ (progressive & fatal)	+ (similar)
In rabbit	- Or mild lesion	+ generalised lesion



Tween-80 hydrolysis test



Niacin test

Nitrate reduction test

RESISTANCE :

- Not heat resistant
- Resistant to chemical disinfectants like phenol
- Destroyed by tincture iodine -5 min
- 80% ethanol – 2-10 minutes
- Sensitive to formaldehyde and glutaraldehyde

VIABILITY :

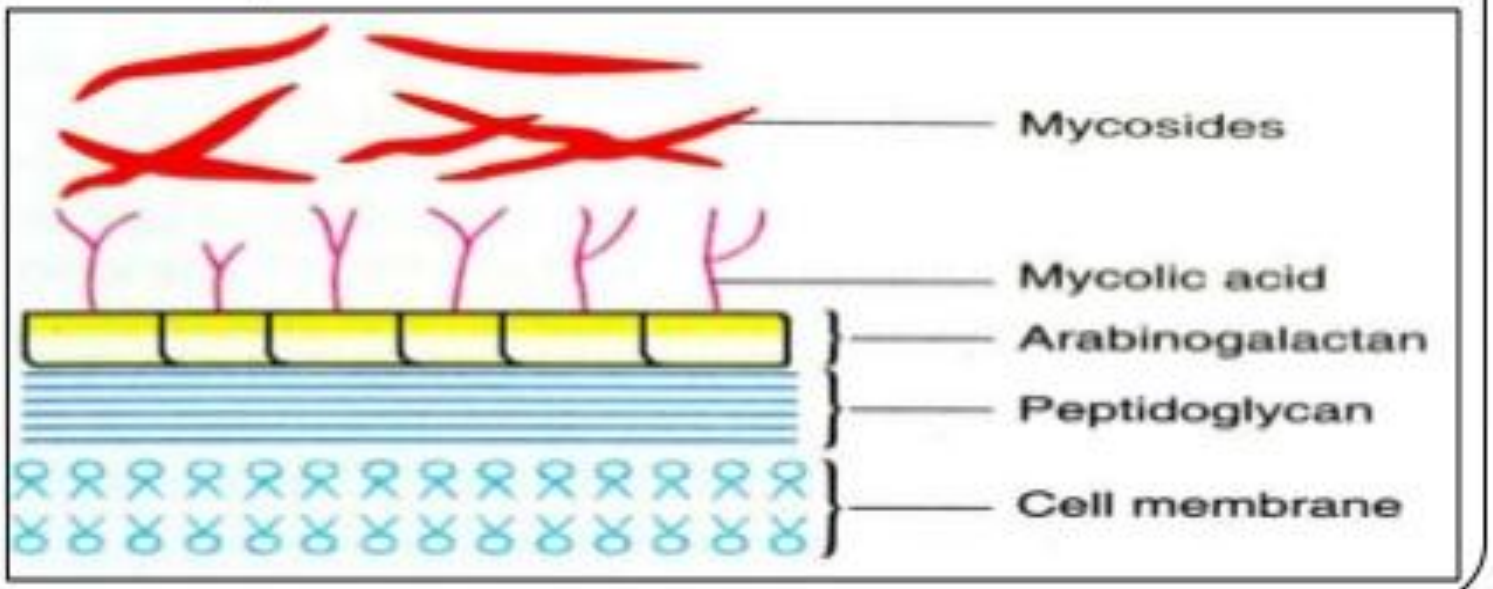
- Sputum – 20-30 hrs
- Droplets - 8-10 days
- Cultures- 6-8 months

Antigenic Structure

- **Cell Wall Antigens:**
  - Peptidoglycan layer
  - Arabinogalactan layer
  - Mycolic acid layer
  - Mycosides

- **Mycolic Acid**
  - Difficult to stain.
  - Difficult to phagocytose.
  - Intracellular survival.
  - Hypersensitivity.
  - Slow growth.
  - Resistant to heat and chemical disinfectants.

- **Cytoplasmic Antigens (Protein antigens)**



Virulence Factor:

- **Cord factor- Trehalose 6-6 dimycolate**, is a glycolipid molecule found in the cell wall of *Mycobacterium tuberculosis* and similar species. It is the primary lipid found on the exterior of *M. tuberculosis* cells.
  - **Serpentine growth** (filaments, cords) grows in close parallel arrangement.
  - Toxic to leukocytes
  - Role in development of granulomatous lesions

- **Sulfolipids-** Sulfated glycolipid (sulfatide) prevent phagosome- lysosome fusion which is important for intracellular survival.

IMMUNITY :

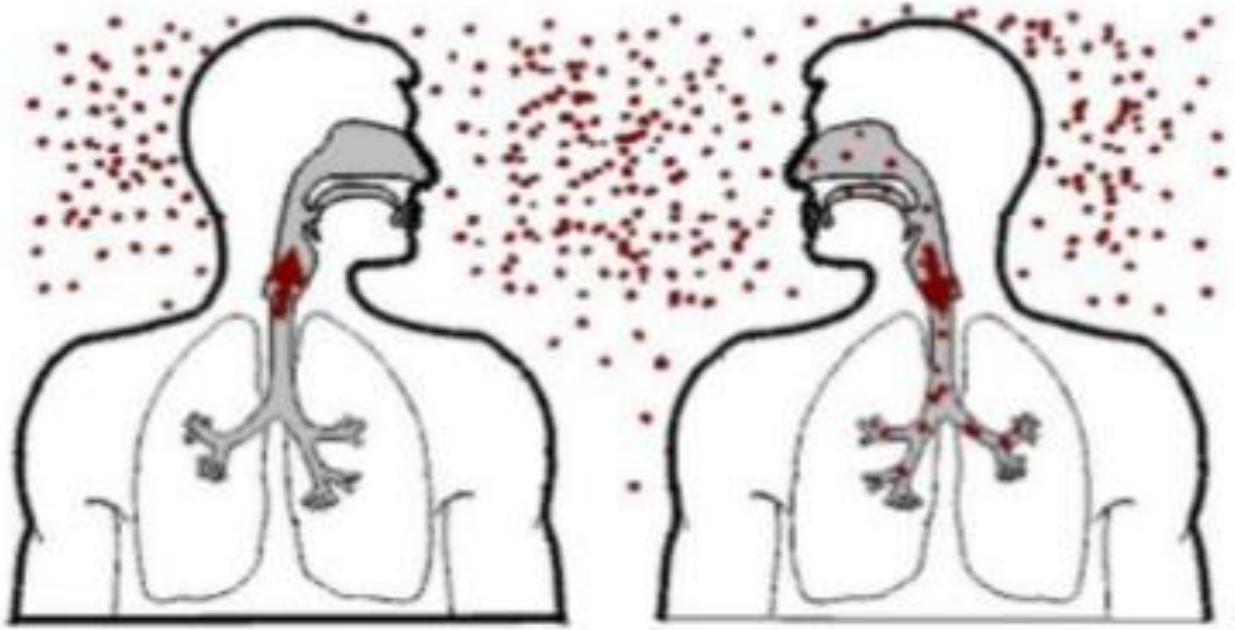
- Following injection by tubercle bacilli, delayed hypersensitivity develops against tuberculoprotein. Antibodies also develop but they don't have any diagnostic value and not relevant in immunity. Immunity in tuberculosis is mainly cell mediated by sensitized T-lymphocytes and macrophages.

- Tubercle Bacilli do not produce any toxin. Various bacterial components have biological effects.

  - **Cell wall** – Causes **Delayed Hypersensitivity**.
  - **Tuberculoprotein** – Induces D.H. Formation of cellular reaction of lymphocytes, monocytes, macrophages, epitheloid cells & giants cells.
  - **Lipids**- Accumulations of macrophages and neutrophils.

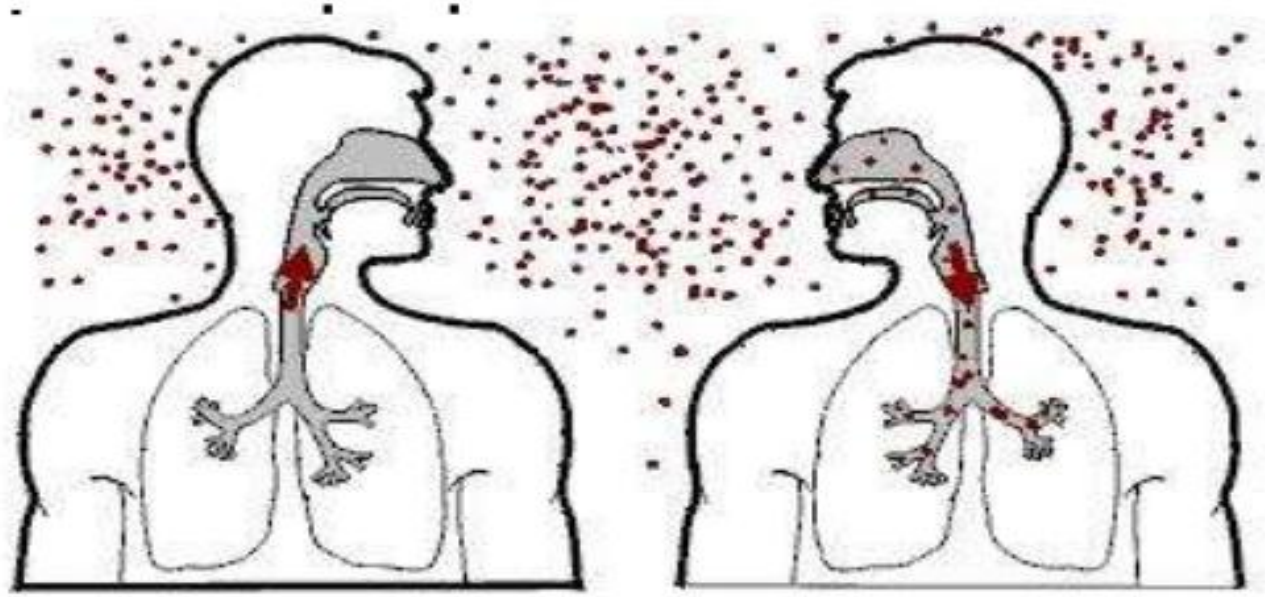
How tuberculosis spreads

- Tuberculosis (TB) is a contagious disease. Like the common cold, it spreads through the air. Only people who are sick with TB in their lungs are infectious. When infectious people cough, sneeze, talk or spit, they propel bacilli into the air. A person needs only to inhale a small number of these to be infected.



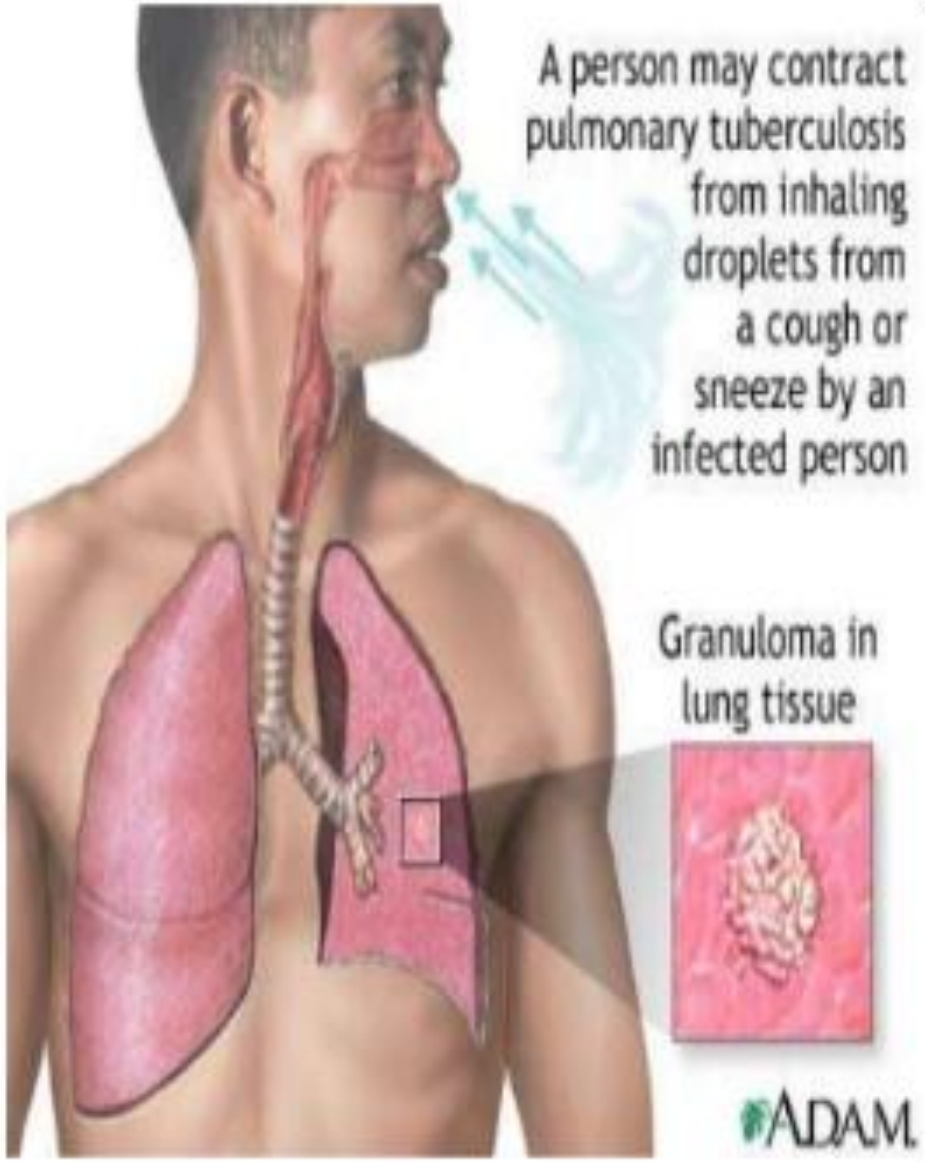
TRANSMISSION

- TB spreads from person to person by airborne



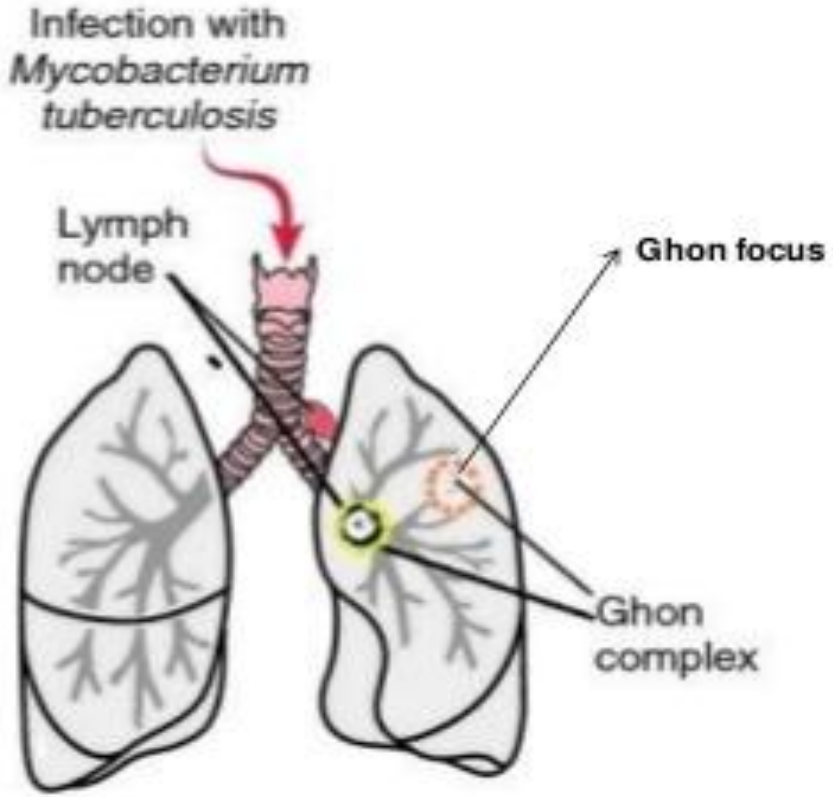
PATHOGENICITY:-

- *M. tuberculosis* can infect any organ or tissue but most commonly lungs are infected; intestines, kidneys, bones, soft tissues, brain etc.
- Infection acquired by inhalation of infected droplets.
- Engulfed by macrophages but survive and multiply.
- Lyses host cell and infect other macrophages.



Primary Tuberculosis:

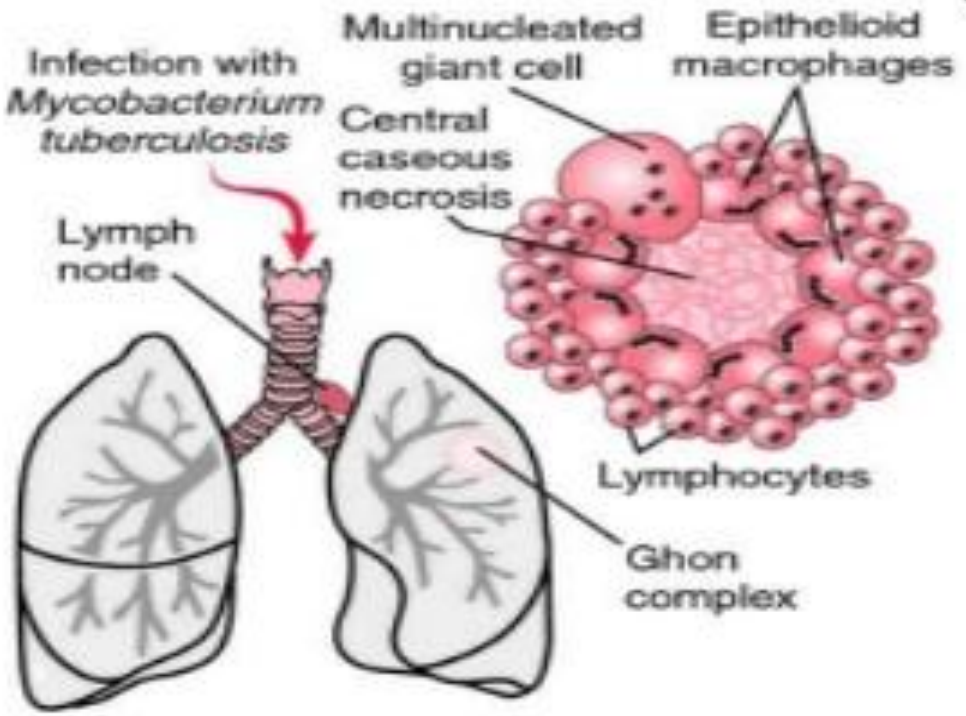
- Mostly asymptomatic.
- Some may have flu like symptoms; chest pain, mild fever and lack of appetite.
- Within 3 weeks, cell mediated immunity checks the bacilli.
- Engulfed bacilli in alveoli forms a lesion called **Ghon focus** in lower lobe. (Anton Ghon, Austrian pathologist)

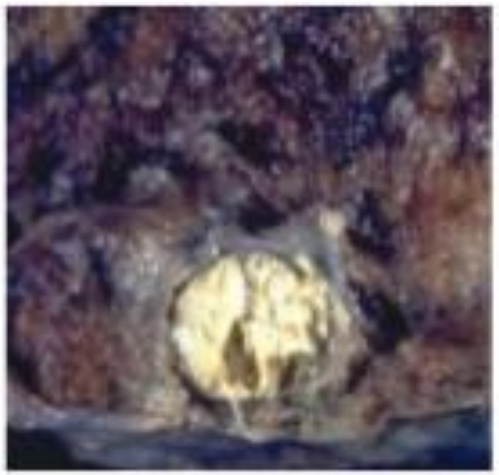


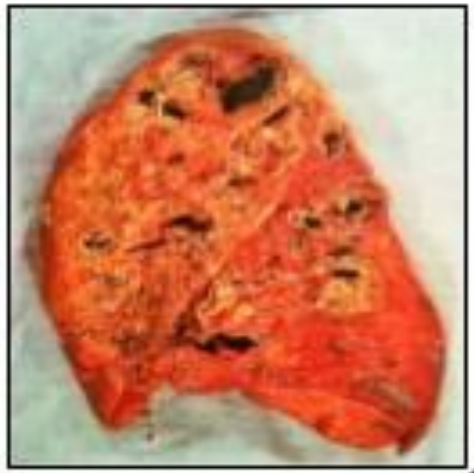
- Some bacilli are transported **to hilar lymph nodes**.
- Ghon focus together with the enlarged hilar lymph nodes is called **Primary Complex (Ghon Complex)**. (Karl Ernst Ranke, German pulmonologist)

Secondary Tuberculosis:

- Caused by reactivation (**immunosuppression**) of the primary lesion.
- Spreads to upper lobes.
- **Granuloma** occurs in apex of lungs.
- Memory T cells releases cytokines.
- Causes tissue destruction and necrosis called **tuberculomas (caeseous necrosis)**.
- Cavities may rupture into blood vessels, spreading bacilli throughout body and in sputum. Causing systemic **Miliary tuberculosis**.

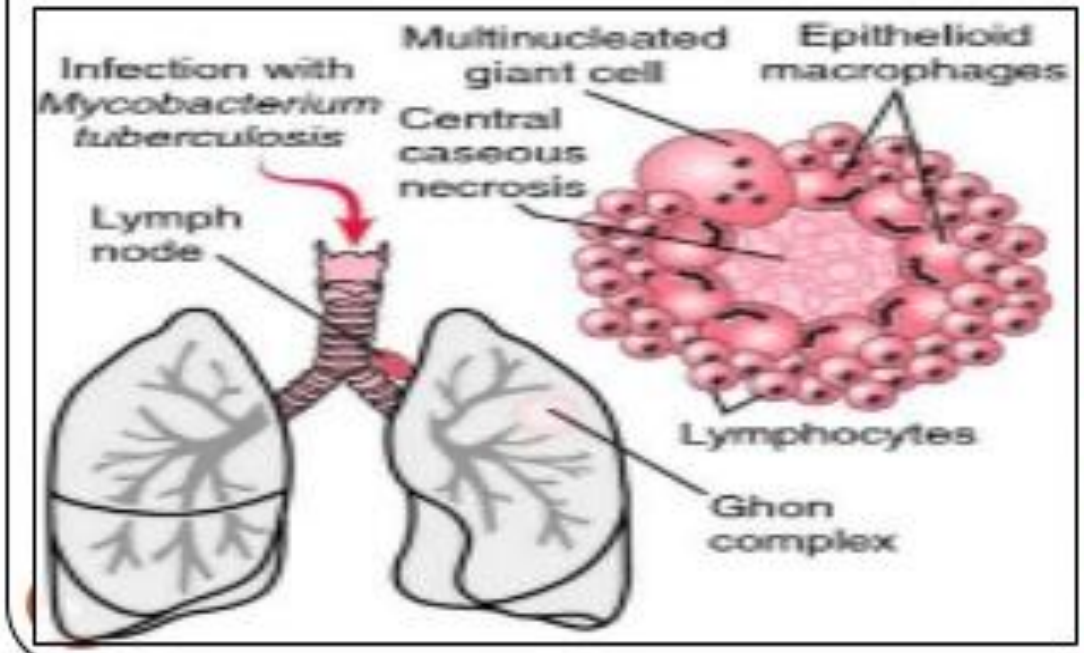


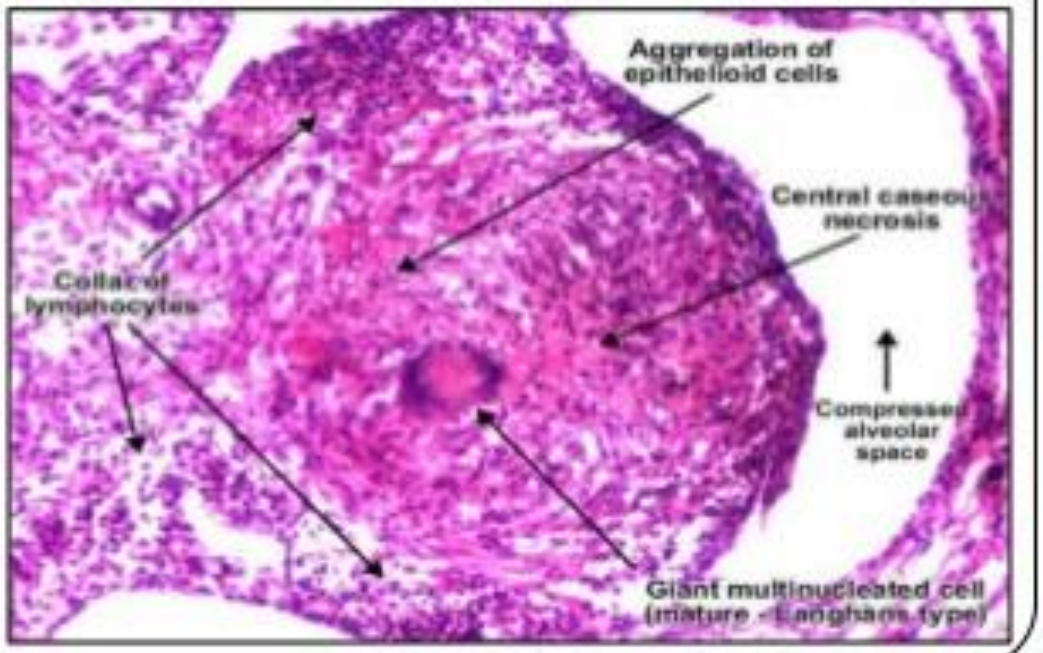




Secondary Tuberculosis: (in 10% cases caused by)

- HIV infection
- Alcoholism and liver cirrhosis
- Malnutrition
- Diabetes
- Steroid and immunosuppressive therapy
- Old age

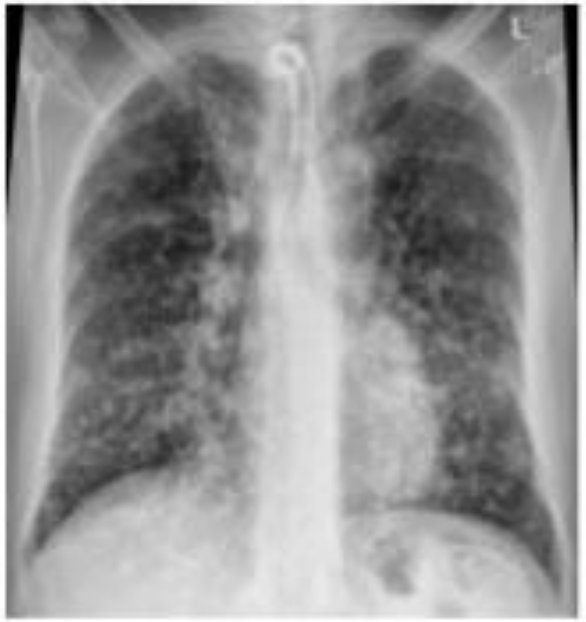


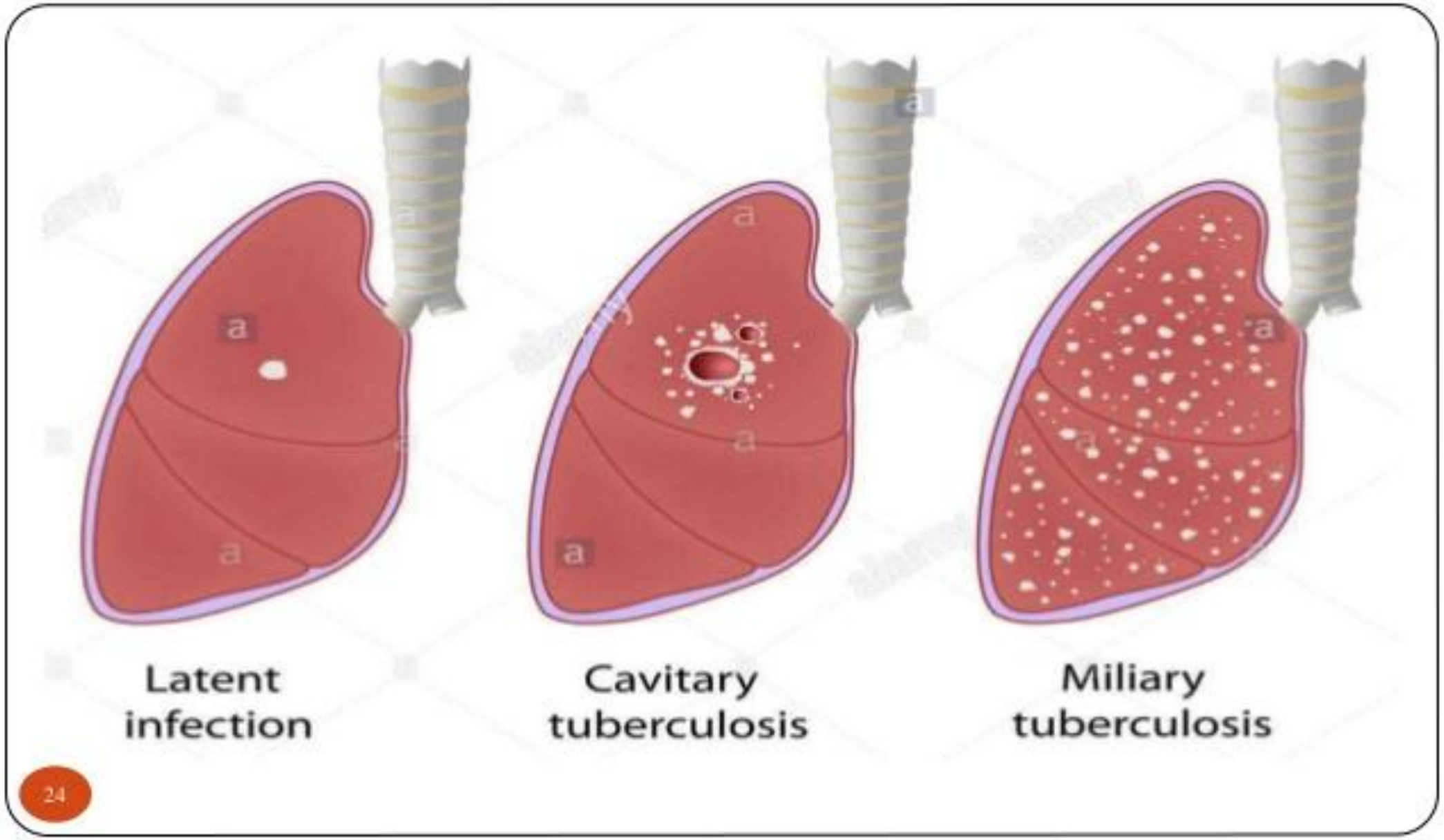


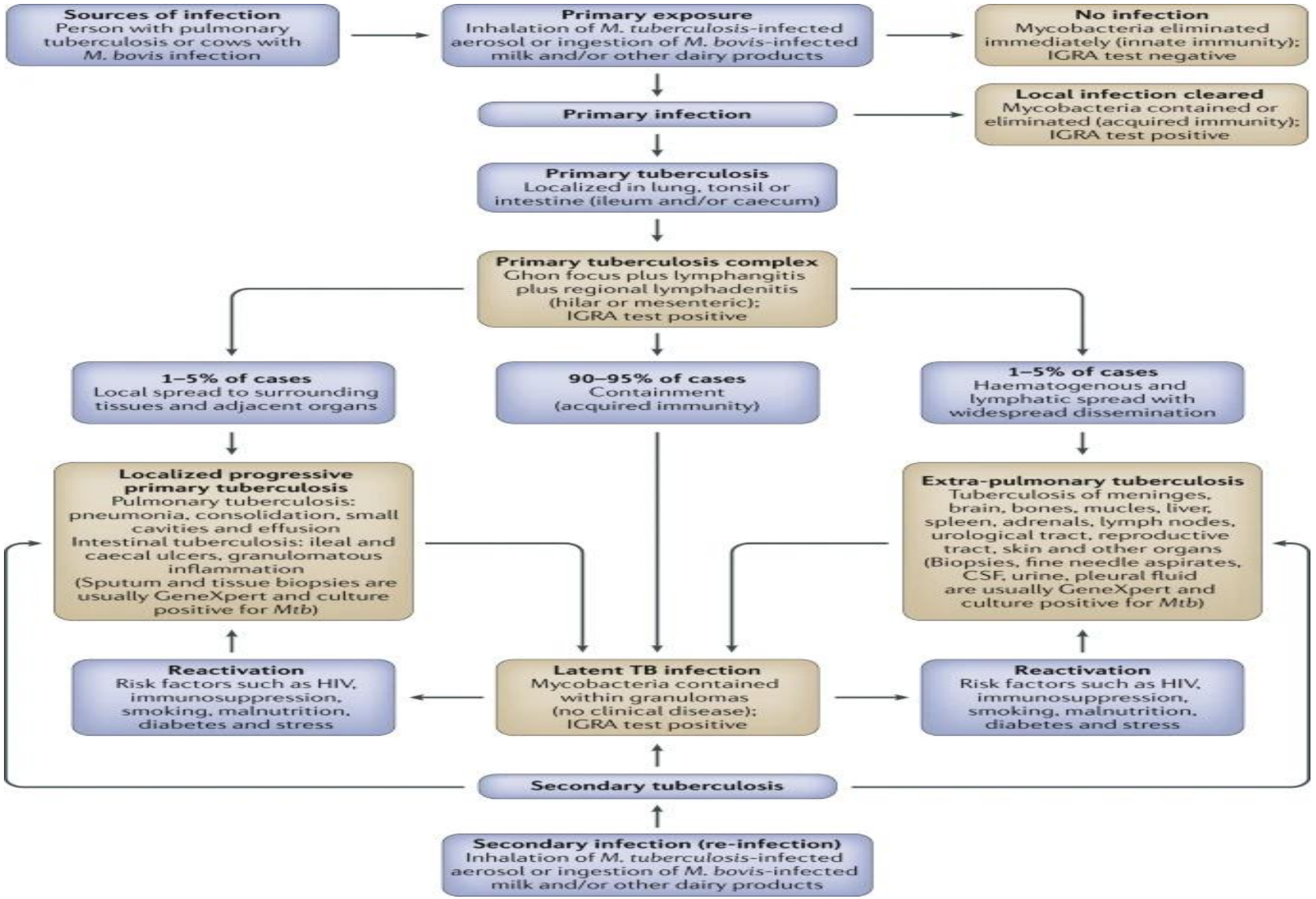
Secondary Tuberculosis:

- Miliary tuberculosis may develop in any organ of the body.
- Certain tissues like heart, striated muscles, thyroid and pancreas are resistant.
- Localization sites are the bone marrow, eye, lymph nodes, liver, spleen, kidneys, adrenal, prostate, seminal vesicles, fallopian tubes, endometrium and meninges.

- **Clinical signs:**
  - Temperature elevation usually in mid-afternoon, night sweats, weakness, fatigability, loss of appetite and weight.
  - Productive cough, blood streaked sputum (hemoptysis)



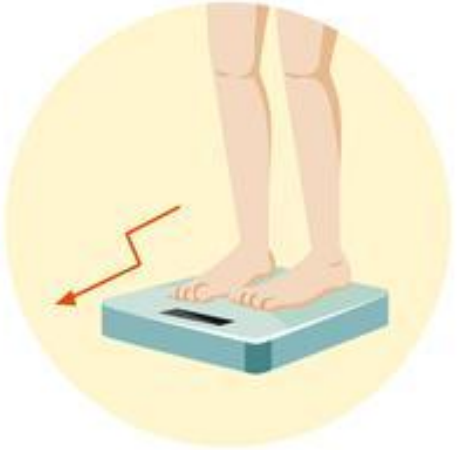

Latent infection
Cavitary tuberculosis
Miliary tuberculosis


Sources of infection
Person with pulmonary tuberculosis or cows with M. bovis infection
Primary exposure
Inhalation of M. tuberculosis-infected aerosol or ingestion of M. bovis-infected milk and/or other dairy products
No infection
Mycobacteria eliminated immediately (innate immunity); IGRA test negative
Primary infection
Local infection cleared
Mycobacteria contained or eliminated (acquired immunity); IGRA test positive
Primary tuberculosis
Localized in lung, tonsil or intestine (ileum and/or caecum)
Primary tuberculosis complex
Ghon focus plus lymphangitis plus regional lymphadenitis (hilar or mesenteric); IGRA test positive
1–5% of cases
Local spread to surrounding tissues and adjacent organs
90–95% of cases
Containment (acquired immunity)
1–5% of cases
Haematogenous and lymphatic spread with widespread dissemination
Localized progressive primary tuberculosis
Pulmonary tuberculosis: pneumonia, consolidation, small cavities and effusion
Intestinal tuberculosis: ileal and caecal ulcers, granulomatous inflammation
(Sputum and tissue biopsies are usually GeneXpert and culture positive for Mtb)
Extra-pulmonary tuberculosis
Tuberculosis of meninges, brain, bones, mucles, liver, spleen, adrenals, lymph nodes, urological tract, reproductive tract, skin and other organs
(Biopsies, fine needle aspirates, CSF, urine, pleural fluid are usually GeneXpert and culture positive for Mtb)
Reactivation
Risk factors such as HIV, immunosuppression, smoking, malnutrition, diabetes and stress
Latent TB infection
Mycobacteria contained within granulomas (no clinical disease); IGRA test positive
Reactivation
Risk factors such as HIV, immunosuppression, smoking, malnutrition, diabetes and stress
Secondary tuberculosis
Secondary infection (re-infection)
Inhalation of M. tuberculosis-infected aerosol or ingestion of M. bovis-infected milk and/or other dairy products

Tuberculosis Symptoms

fever

fatigue



weight loss

persistent cough

blood in cough

night sweats

LAB DIAGNOSIS:-

- **Specimen** depending on clinical presentation –Sputum, Pus, Urine, CSF, Pleural/ Ascitic fluid.
- **Pulmonary tuberculosis** - Early morning sputum sample on 3 consecutive days. (Bacillary shedding is intermittent).
- Sputum is collected in wide mouth containers.

PROCESSING

Physical exam

Quantity

Colour

Consistency

Presence of blood

Direct smear stained by Z.N. method

Conc. Petroff's method

Smear

Culture

Interpretation of sputum stained by Z - N Stain (WHO)

More than 10 bacilli / field ---------- +++

From 1 – 10 bacilli / field ---------- ++

From 10 – 99 bacilli / 100 fields ---------- +

From 1 -9 bacilli/100 fields ---------- write the exact no.

No bacilli seen ---------- NEGATIVE

***(10,000 bacilli / ml of sputum): shows positive**

TEST PRINCIPLES :-

1. **Niacin test-** suspension of tubercle bacilli +10% cyanogen bromide 4% aniline in ethanol- positive gives yellow colour. (+MTB)

2. **Arylsulphatase test-** Bacteria grown in solution of disulphate + 2N NaOH – pink (-MTB)

3. **Neutral Red Test** – colonies of Tub. Bacilli in neutral red solution in alk buffer –colonies pick up red colour (+MTB)

4. **Catalase Peroxidase test-** 5ml culture suspension + H_2O_2 and 2% catechol – effervescence - Catalase Peroxidase positive. Point mutation is a catalase gene, makes the strains resistant to isoniazide. (weakly + MTB)

5. **Amidase test-** Acetamide, benzamide, carbamide, nictonamide, pyrizinamide. (Split)
   - 0.00164M solutions of amide + tub. bacterial suspension incubate at 37°c.
   - Add solution of phenol, $MnSo_4$, hypochlorite.
   - Boil tube for 20 mins.
   - Blue colour indicates – Positive reaction

Drug sensitivity tests: -

1. **Absolute conc. Method**:-

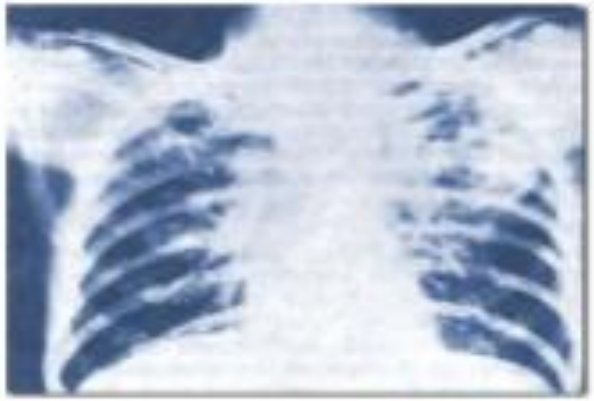
   L.J. media containing serial conc. Of drug are inoculated & minimum inhibitory conc. Noted.

2. **Resistance ratio**:-

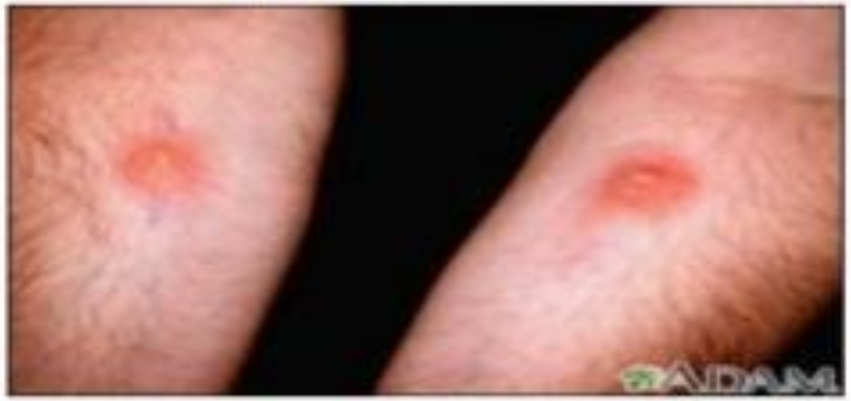
   Two sets of media containing serial conc. Of drugs are inoculated.

   - 1st set – test strain
   - 2nd set – standard strain

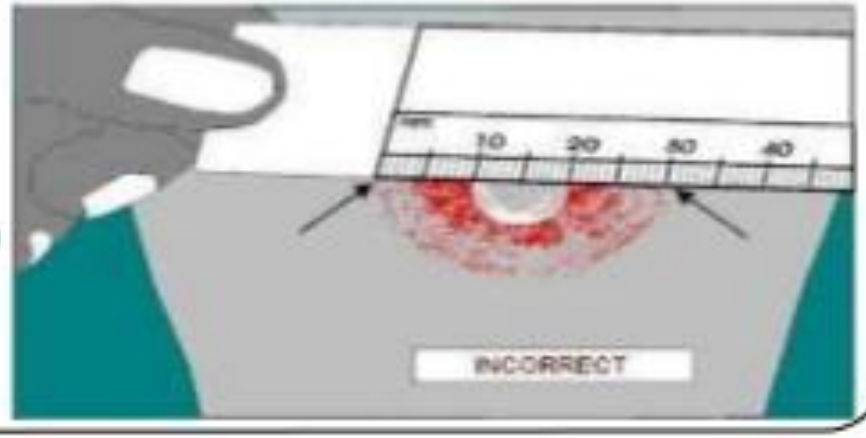
OTHER METHODS OF DIAGNOSIS OF TUBERCULOSIS:-



1. **X-ray chest**
2. **Blood exam** – lymphocytosis, increased ESR
3. **Mantoux test** – Tuberculin test.



- Routinely 5TU is used. 0.1 ml of PPD is injected intradermally in forearm. The area is marked by pen do not press or wash.
- Readings taken after 48-72 hrs.
- Erythema & indurations > 10mm – positive
  < 5mm – negative (+ in HIV)
  6-9mm – equivocal



1

Result Interpretation

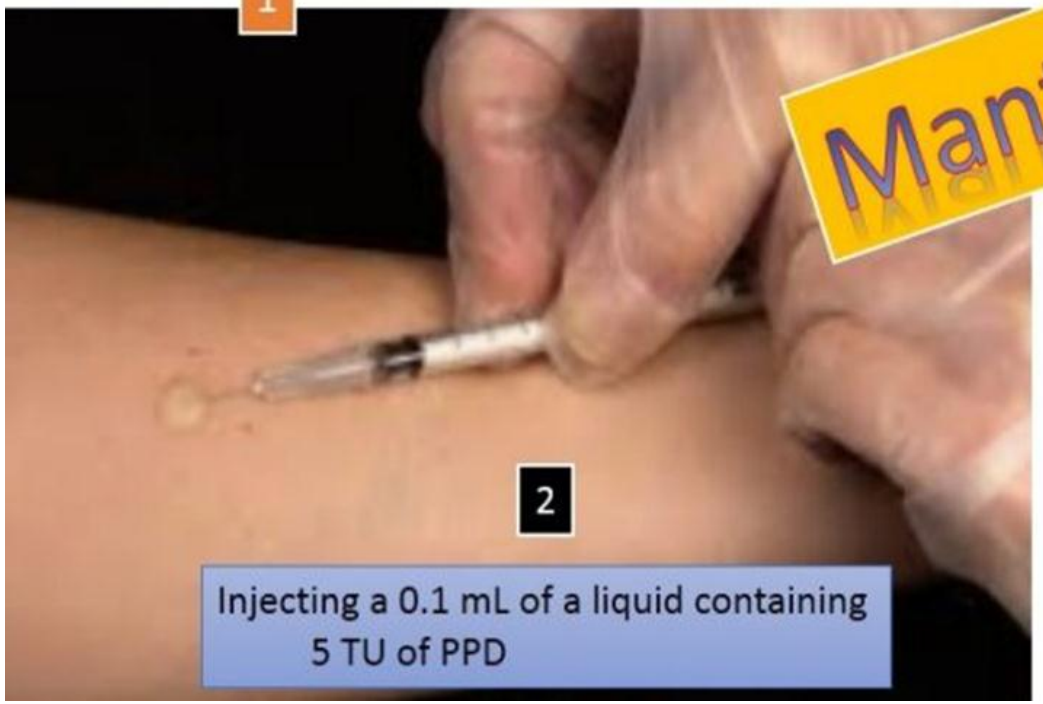
Negative: Induration < 5 mm (always)

Positive: Induration ≥ 5 mm not always but conditional e.g. in Immunosuppressed persons

10 mm induration to be positive e.g. IV drug abusers, children under 4 years old, people in high risk areas

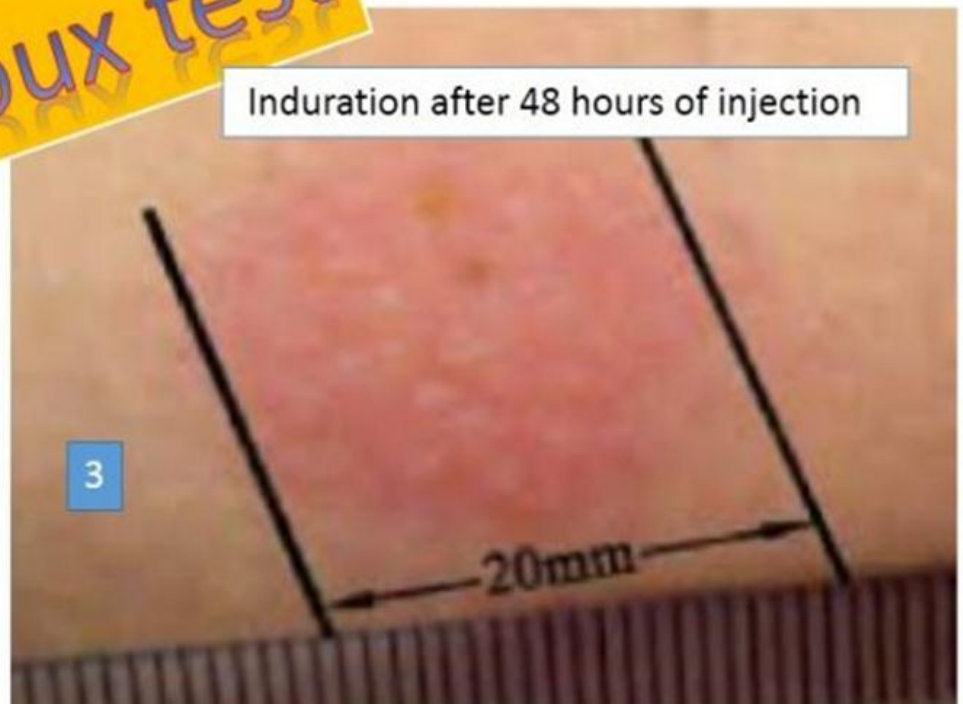
≥ 15 mm induration to be positive in a healthy person whose immune system is normal

Mantoux test



2

Injecting a 0.1 mL of a liquid containing 5 TU of PPD



3

NEWER METHODS FOR LAB DIAGNOSIS OF TUBERCULOSIS:-

1. **Radiometric methods** –

Advantage:- rapid growth

- Specific identification,
- Result within 7 days

Instrument:-

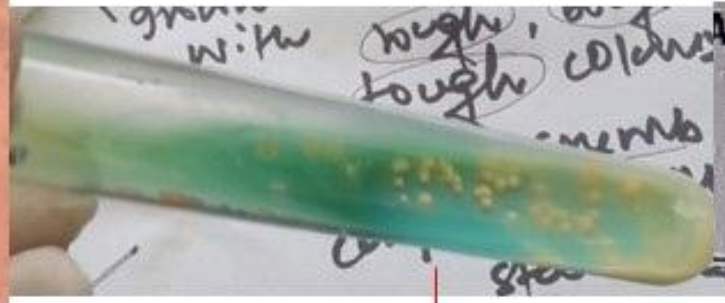
- **BACTEC**
- Fully automated

2. **PCR** – high sensitivity.
   - DNA amplified.
   - Cannot differentiate living and dead bacteria; both reported positive.

3. **Serology** – antibodies against *M.tubercule* antigen by ELISA.

Stain

Culture



PCR

FOR POSITIVE SAMPLE

Sputum smear minimum contain 10000 bacilli /ml

Sputum culture 10-100 bacilli/ml

PCR-load should be 1-10 bacilli/ml

Mycobacterium tuberculosis

TREATMENT

FIRST LINE DRUG:-

- Rifampicin(R) & Pyrizinamide (Z) – kill bacilli in lesions
- Isoniazid (H) – kills replicating bacilli
- Streptomycin (S) – kills extracellular bacilli
- Ethambutol (E) – bacteristatic

- **Intensive phase** – 3 times a week, 2 months – H, E, R, Z
- **Continuing phase** – 3 times a week, 4-5 months – H, R

SECOND LINE DRUG:-

- Quinolones, Aminoglycosides, Macrolides, Thiacetazone, Cycloserine, Capneomycin.

- **MDR-TB** – Resistance to Rifampicin & Isoniazid ; **DOTS** (directly observed therapy under supervision) important.

TYPES OF DRUG RESISTANCE

Mono resistance: resistant to one drug

Poly resistance: resistant to 2 or more drugs

Multidrug-resistant tuberculosis (MDR-TB)- resistant to at least isoniazid and rifampin

Pre- XDR TB – MDR TB + resistance to a fluoroquinolone or a 2nd line injectable drug

Extensively Drug Resistant TB (XDR TB) – MDR-TB + resistance to a fluoroquinolone and a second line injectable (amikacin/ kanamycin/capreomycin)

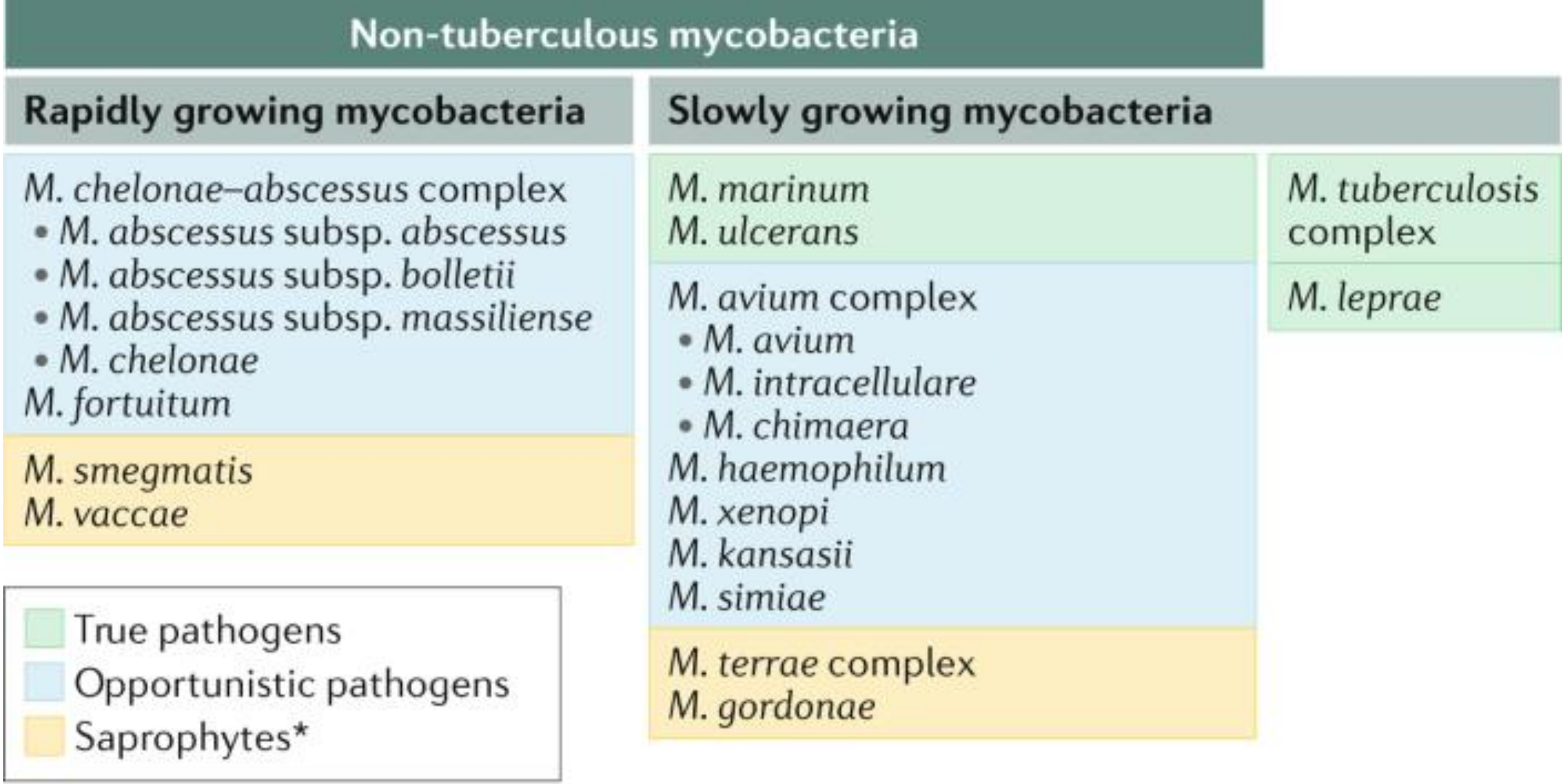
Totally Drug Resistant TB (TDR-TB) ???

BACILLUS CALMETTE GUERIN (BCG) :–

- Live attenuated vaccine. Strain of ***M. bovis*** attenuated by serial sub cultures in glycerine bile potato medium over 13 years.
- 0.1ml injected intradermally on deltoid muscle soon after birth.
- Immunity last for about 15 years.

BCG not to be given –

- Infants & children with active HIV disease.
- Babies born to sputum AFB positive mother.

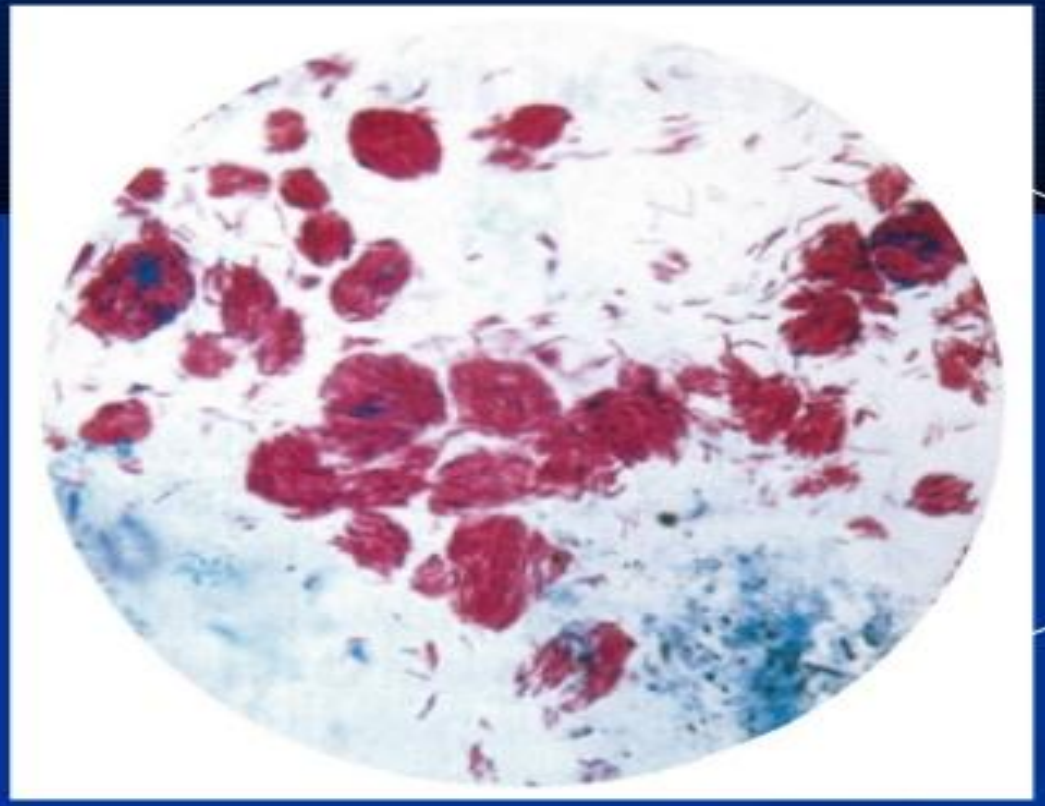

Non-tuberculous mycobacteria
Rapidly growing mycobacteria
Slowly growing mycobacteria
M. chelonae–abscessus complex
• M. abscessus subsp. abscessus
• M. abscessus subsp. bolletii
• M. abscessus subsp. massiliense
• M. chelonae
M. fortuitum
M. smegmatis
M. vaccae
M. marinum
M. ulcerans
M. avium complex
• M. avium
• M. intracellulare
• M. chimaera
M. haemophilum
M. xenopi
M. kansasii
M. simiae
M. terrae complex
M. gordonae
M. tuberculosis complex
M. leprae
True pathogens
Opportunistic pathogens
Saprophytes*
*can be detected in clinical samples and need retesting to confirm infection

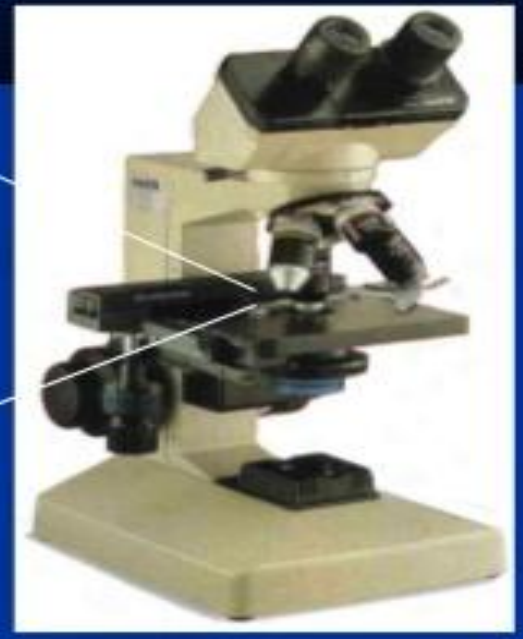
Leprosy

Lepra bacilli

- Gram positive Obligate intracellular bacillus - due to its large pool of non functional genes.
- Acid fast stained with modified Fite stain or ZN stain
- Short, thick, pink stained rods of Size: (5μ X 0.5 μ)
- Occurs characteristically in clumps or bundles("globi")
- Affinity for Schwann cells & cells of R-E system .
- *M. leprae* grows best in cooler tissues (the skin, peripheral nerves, anterior chamber of the eye, upper respiratory tract, and testes), sparing warmer areas of the skin (the axilla, groin, scalp, and midline of the back).
- Optimal temp. for growth is 30-33 centigrade

The Leprosy Bacteria





Cultivation

- *M. leprae* is found only in cases of human infection.
- They have not yet been grown ion artificial media or tissue culture.
- The generation time of leprae bacillus is found to be 12-13 days on an average.
- When ground tissue or nasal scrapping from lepromatous leprosy containing lepra bacilli are inoculated intradermally into foot pad of mouse and kept at low temperature (20°C), a granulomatous lesion develops at the site of injection in 1-6 months.
- When nine band armadillo is inoculated with lepra bacilli, generalized infection develops with extensive multiplication of the bacilli.

Reservoir of infection

- **Main reservoir :** Human being
  - Lepromatous case> Non lepromatous cases

- **Animal reservoirs**
  - 9-banded armadillos
  - Chimpanzees
  - Mangabey monkeys
  - Sphagnum moss

Mode of transmission

- **Transmission by inhalation**
  - Droplet infection(most common)
- **Transmission by contact**
  - Skin to skin contact with infectious cases
  - Contact with soil or fomites
- **Other Routes**
  - Insect Vectors e.g.. Mosquito, Bedbugs
  - Tattooing needles

NB : Breast feeding and Transplacental infection do not occur.

Incubation period

- **Long incubation period**
  - Ranged: 2 to 40 years or more
  - Average: 3-5 years
- **Generation time** : 12 days.
- **Infectivity** : Leprosy is a highly infectious disease with low pathogenicity. Among household contacts of lepromatous cases about 5 to 12 percent is expected to show signs of leprosy within 5 yrs.

VIRULENCE FACTOR

The bacterium's complex cell wall contains large amounts of an *M. leprae*–specific **phenolic glycolipid (PGL-1),** which is detected in serologic tests. The unique trisaccharide of *M. leprae* binds to the basal lamina of Schwann cells; this interaction is probably relevant to the fact that *M. leprae* is the only bacterium to invade peripheral nerves.

Bacilli discharged from nose

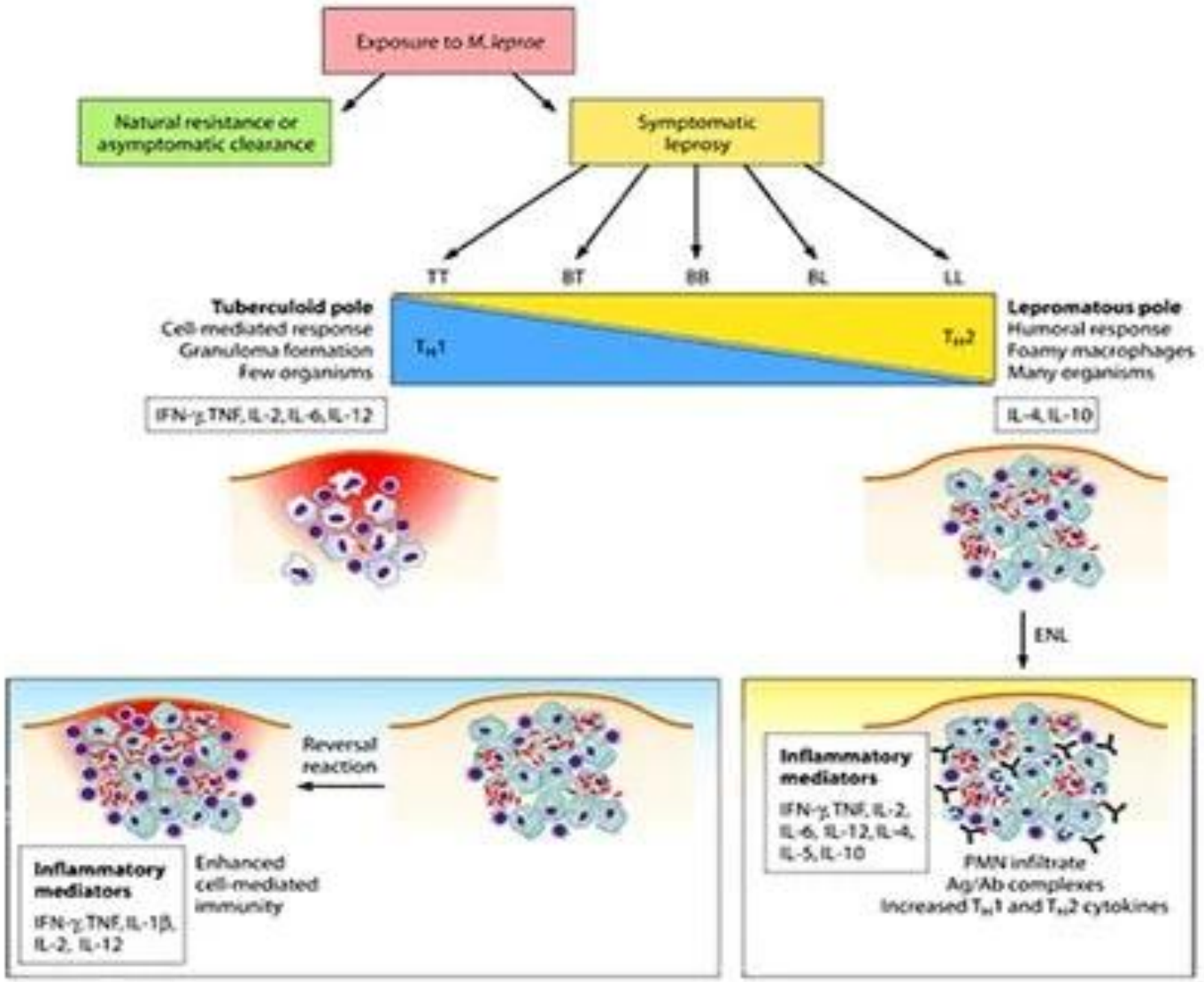
Inhaled by susceptible person

Taken up by alveolar macrophages

Disseminated through blood

Spreads to nerve and skin

Bacilli proliferate especially in Schwann cells



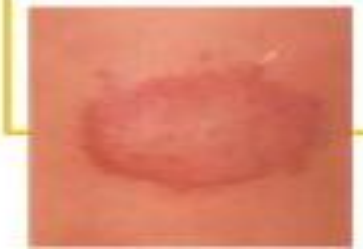
Ridley- Jopling 1966 (Research purposes)

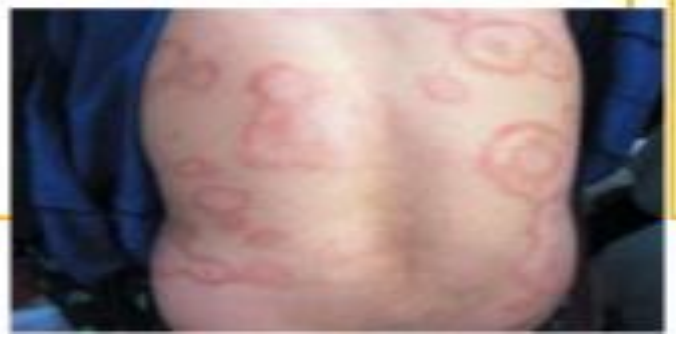
- **Most widely accepted**
  - Divides Leprosy cases into five groups according to their position on an immunohistological scale.
  - It can be used only when full research facilities are available :
    - Tuberculoid (TT)
    - Borderline Tuberculoid (BT)
    - Borderline Borderline (BB)
    - Borderline Lepromatous (BL)
    - Lepromatous (LL)

Differences

Tuberculoid Leprosy (TT)

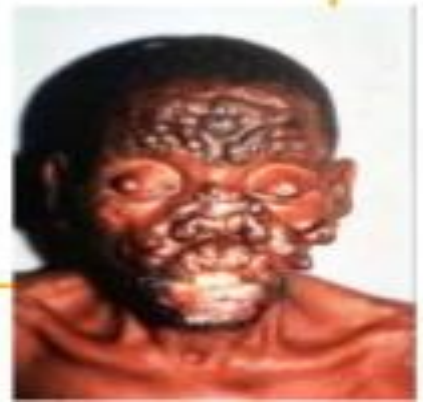
- Well demarcated, dry patch
- Minimal disfigurement
  - No leonine facies
  - No claw-shaped hands
  - No pendulous ear lobes
- Good immune response (high resistance)



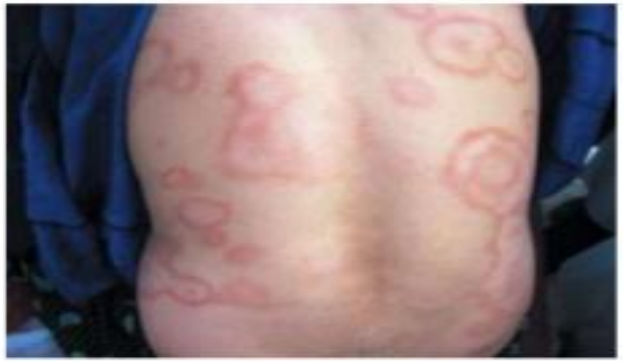


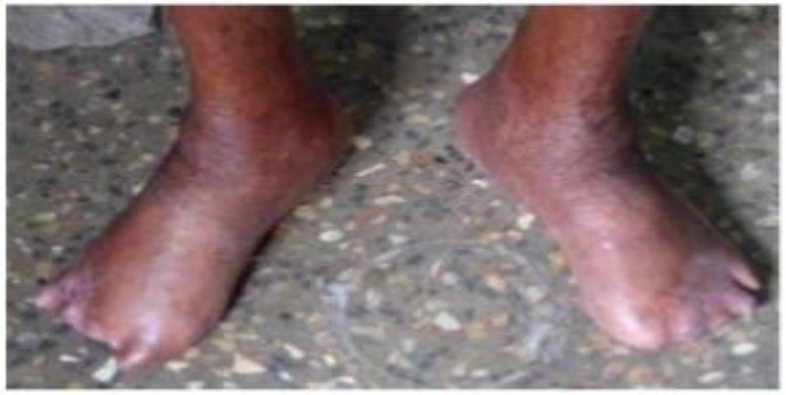
Lepromatous Leprosy (LL)

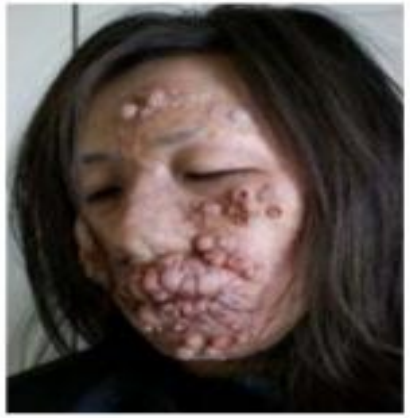
- Disfigurement is there
  - Leonine facies
  - Claw-shaped hands
  - Pendulous ear lobes
  - Saddle nose
- Suppressed (low resistance)

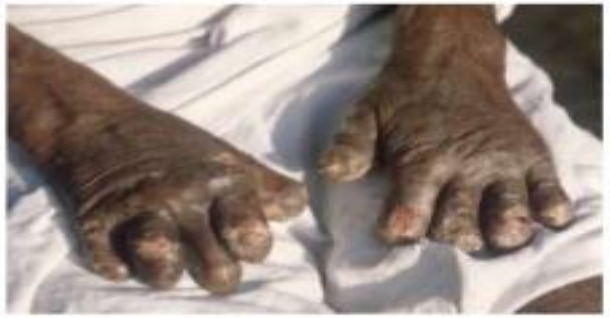


Leprosy









Clinical and Immunologic Manifestations of Leprosy

Features	Tuberculoid Leprosy	Lepromatous Leprosy
Skin lesions	Few erythematous or hypopigmented plaques with flat centers and raised, demarcated borders; peripheral nerve damage with complete sensory loss; visible enlargement of nerves	Many erythematous macules, papules, or nodules; extensive tissue destruction (e.g., nasal cartilage, bones, ears); diffuse nerve involvement with patchy sensory loss; lack of nerve enlargement
Histopathology	Infiltration of lymphocytes around center of epithelial cells; presence of Langhans cells; few or no acid-fast rods observed	Predominantly "foamy" macrophages with few lymphocytes; lack of Langhans cells; numerous acid-fast rods in skin lesions and internal organs
Infectivity	Low	High
Immune response	Delayed hypersensitivity reactivity to lepromin	Nonreactivity to lepromin
Immunoglobulin levels	Normal	Hypergammaglobulinemia
Erythema nodosum	Absent	Usually present

DIAGNOSIS

BACTERIOLOGICAL EXAMINATION

This includes :

- Skin Smears :
- Nasal Smears or blows :
- Nasal Scrapings :

DIAGNOSIS

BIOPSY

- Usually resorted to when there is high clinical suspicion but the other test are unyielding. It also gives information about the bacterial content of skin.

DIAGNOSIS

IMMUNOLOGICAL TESTS

- **Tests for cell mediated immunity(CMI)**
  - **LEPROMIN TEST**

- **Tests for humoral antibodies(serological tests)**
  - **FLA-ABS test** : used for detecting subclinical infections. **92.3** percent sensitive and **100** percent specific in detecting specific antibodies in all types leprosy irrespective of type and duration of disease.
  - Monoclonal antibodies
  - Others : RIA, ELISA.

DIAGNOSIS

LEPROMIN TEST

Method : it is performed by injecting 0.1ml of lepromin into inner aspect of the forearm. The reaction is read at 48 hours and 21 days. Two types of reaction have been described :

EARLY REACTION(FERNANDEZ REACTION) :

an inflammatory reaction develops within 24 to 48 hours and this tends to disappear in 3 to 4 days. If the diameter of the red area is more than 10mm the test is considered positive. It is a delayed type hypersensitivity reaction to soluble constituents of lepra bacilli and indicates whether or not a person has been sensitized by exposure to and infection by lepra bacilli.

DIAGNOSIS
LEPROMIN TEST

LATE REACTION(MITSUDA REACTION) : It is characterized by the appearance of a nodule which becomes apparent in 7 to 10 days and reaches its maximum in 3 to 4 weeks. The test is read at 21 days. If the nodule is more than 5 mm it is considered positive. It is induced by the bacillary component and indicates cell mediated immunity.

- In the first six months of life most children are lepromin negative
- BCG vaccination is capable of converting lepra reaction from negative to positive.

DIAGNOSIS
LEPROMIN TEST

VALUE OF LEPROMIN TEST :

- Useful tool for evaluating the immune status of leprosy patients.
- Aid to classify the type of disease.
- Estimating the prognosis
- Strongly positive in a typical tuberculoid case and getting weaker towards the lepromatous end, the typical lepromatous case being lepromin negative indicating failure of CMI.

The greatest drawback being high false positive and false negative cases hence not used as a diagnostic test.

OTHER TESTS FOR CMI :

- Lymphocyte transformation test(LTT)
- Leucocyte migration inhibition test(LMIT)

Treatment

- **Multiple drug therapy** for 12 – months is key to treatment, this is carried out by WHO guideline using.
  1- Rifampicin
  2- Dapsone
  3- Clofazimine

- During treatment, patient may develop acute manifestation, which controlled by **steroids**

- **Surgical treatment** is indicated in advance stage of disease for functional disability of limbs, cosmetic disfigurement of face and visual problems.

- Surgical reconstruction requires the expertise of hand surgeon, orthopedic surgeon and plastic surgeon.

Actynomyces - Taxonomy

(Domain): Bakteriyalar

(Kingdom): Actinomycetota

(Class): Actinomycetia

(Order): Actinomycetales

(Family): Actinomycetaceae

(Genus): Actinomyces

(Species): ***A.israelli***

Actinomycetes

- Fungus-like characteristics
  - Branching filaments in tissues / culture
  - looks like mycelia
- Filaments frequently segmented
  - Pleomorphic forms (Diphtheroid & club shaped)
- Cell wall and the internal structures are typical of bacteria.
- Aaerobic OR Anaerobic.
- Slow growers

Actinomycetes

- Classification
  - Anaerobic
    - Actinomyces spp
  - Aerobic
    - Nocardia spp
    - Actinomadura spp
    - Streptomyces spp

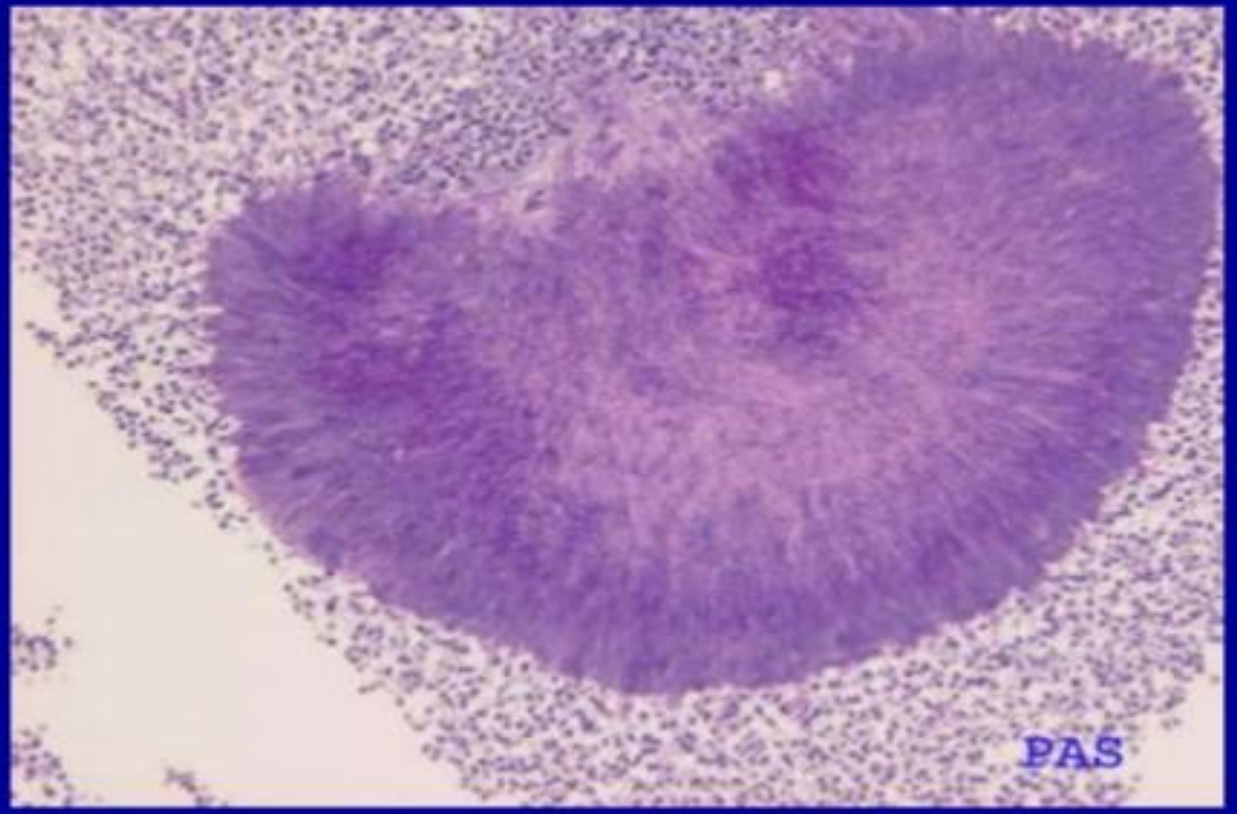
Actinomyces

- Anaerobic Actinomycetes
  - Morphology and cultural characteristics
    - Gram positive branching, or diphtheroid-like bacilli
    - Anaerobic and require CO_2 for growth
    - Non-sporing
    - Grows well on Blood Agar.

Actinomycosis

- Characterized by draining sinuses,
- containing characteristic granules
  - which are micro colonies of bacteria
  - look like dense rosettes of club-shaped filaments in radial arrangement
- Ray fungus

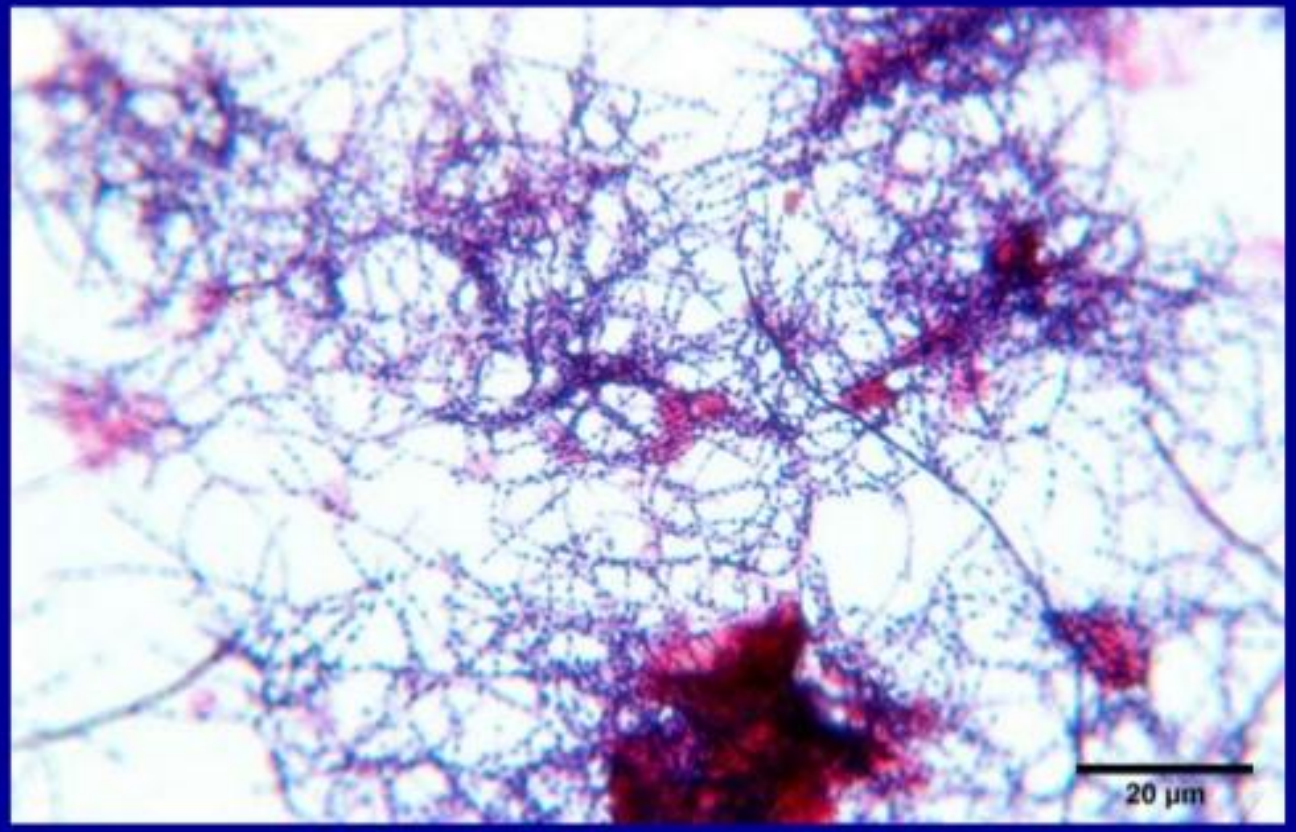
Ray fungus



Actinomyces

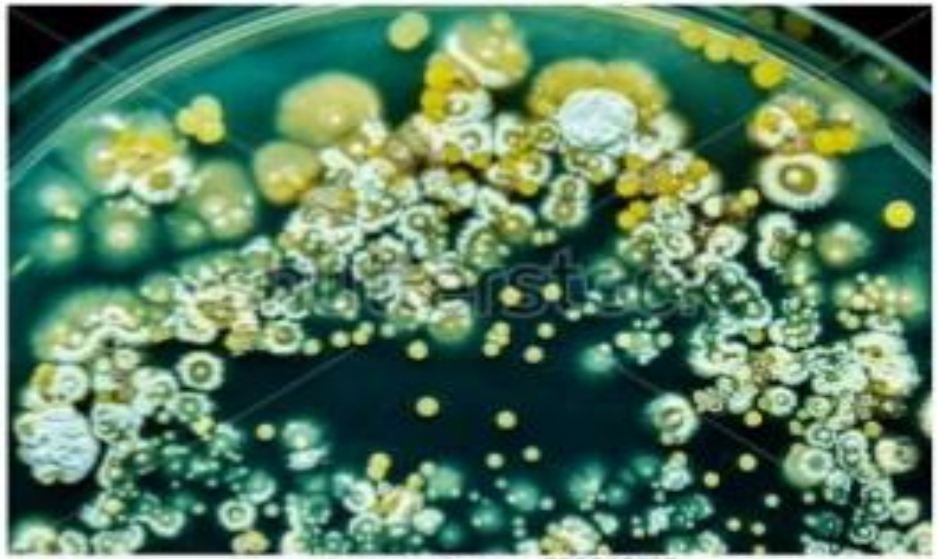
- Clinical significance
  - Part of the normal oral bacterial flora in humans and animals.
  - Three clinical types
  - Cervico facial actinomycosis or "lumpy jaw"
    - » occur following tooth extractions or dental surgery
    - » rare today because of prophylactic antibiotic therapy
  - Thoraco Lumbar actinomycosis
  - Abdominal actinomycosis
  - Meningitis, endocarditis, or genital infections

Actinomyces – Gram stain



Actinomycetes - Gram staining

Acrtinomycetes - culture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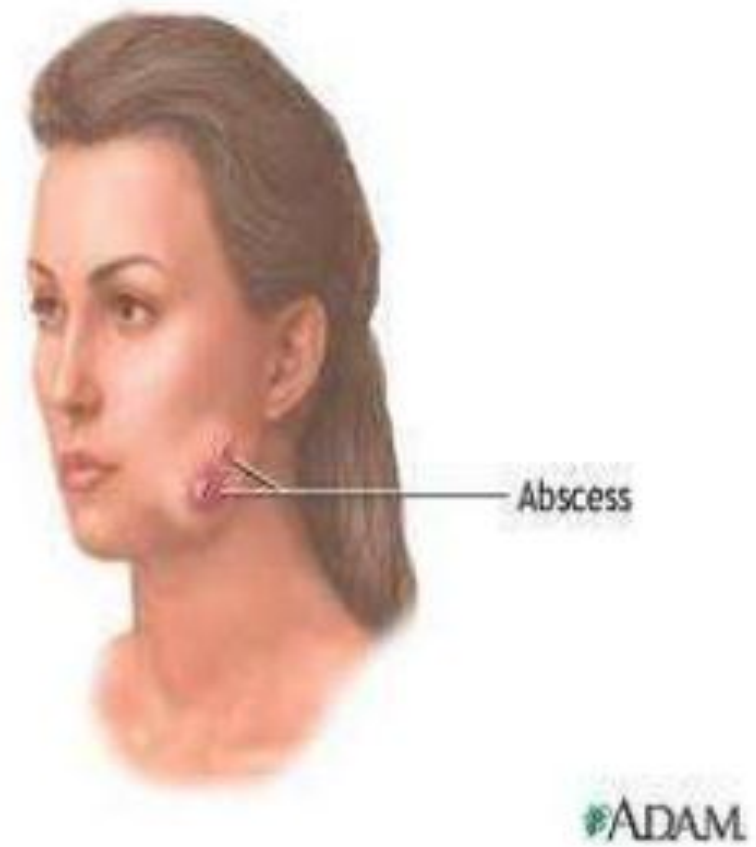
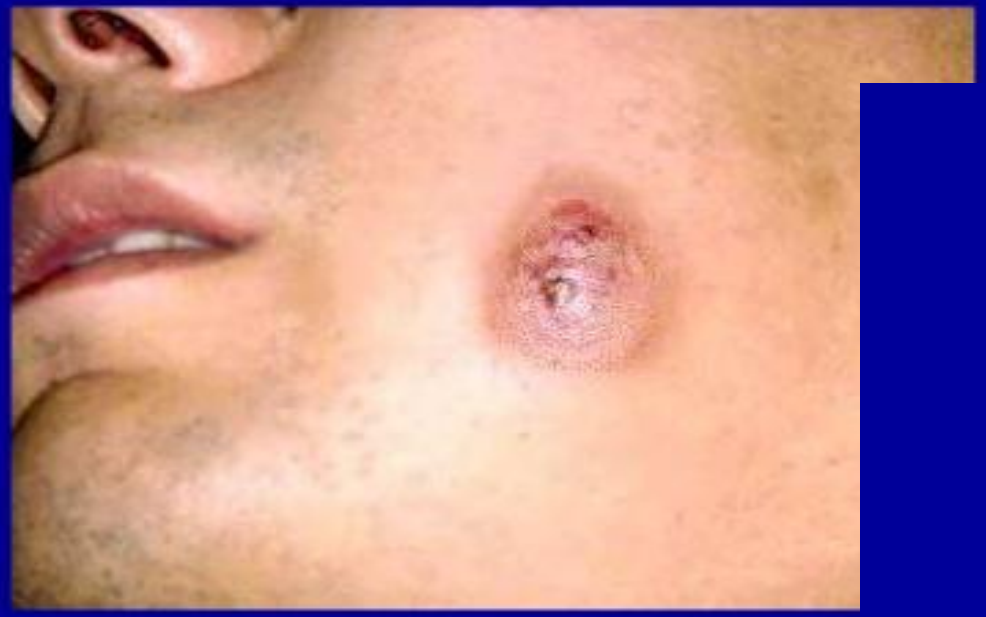
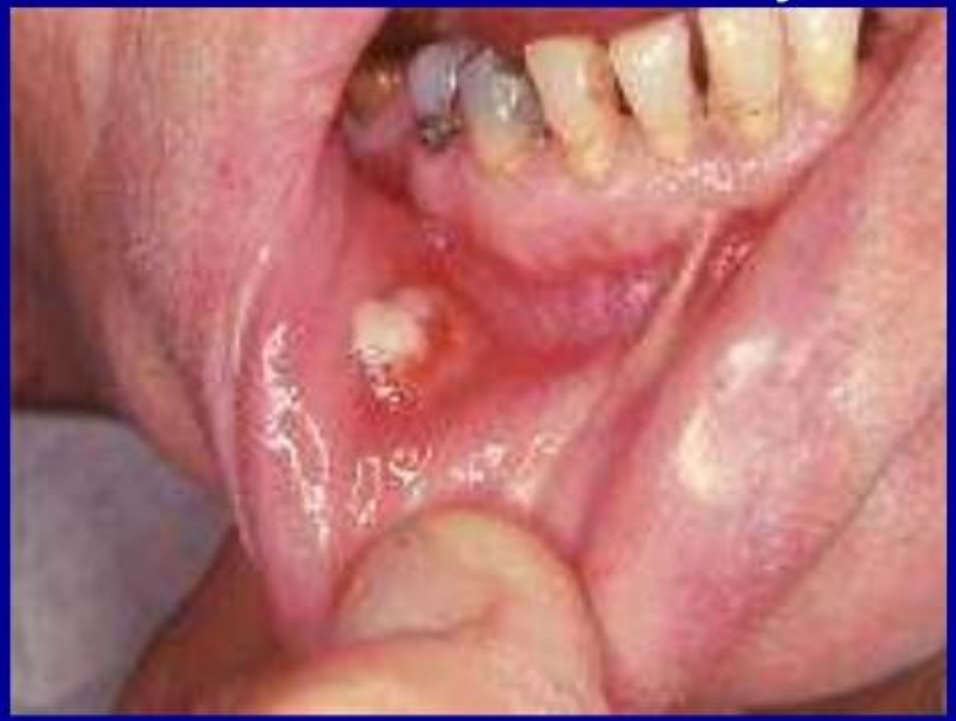
Clinical presentation



Cervicofacial Actinomycosis



Cervicofacial Actinomycetes



Diseases of Selected Pathogenic Actinomycetes

Organism	Diseases	Frequency
Nocardia	Pulmonary diseases (bronchitis, pneumonia, lung abscesses); primary or secondary cutaneous infections (e.g., mycetoma, lymphocutaneous infections, cellulitis, subcutaneous abscesses); secondary central nervous system infections (e.g., meningitis, brain abscesses)	Common
Rhodococcus	Pulmonary diseases (pneumonia, lung abscesses); disseminated diseases (e.g., meningitis, pericarditis); opportunistic infections (e.g., wound infections, peritonitis, traumatic endophthalmitis)	Uncommon
Gordonia	Opportunistic infections	Rare
Tsukamurella	Opportunistic infections	Rare

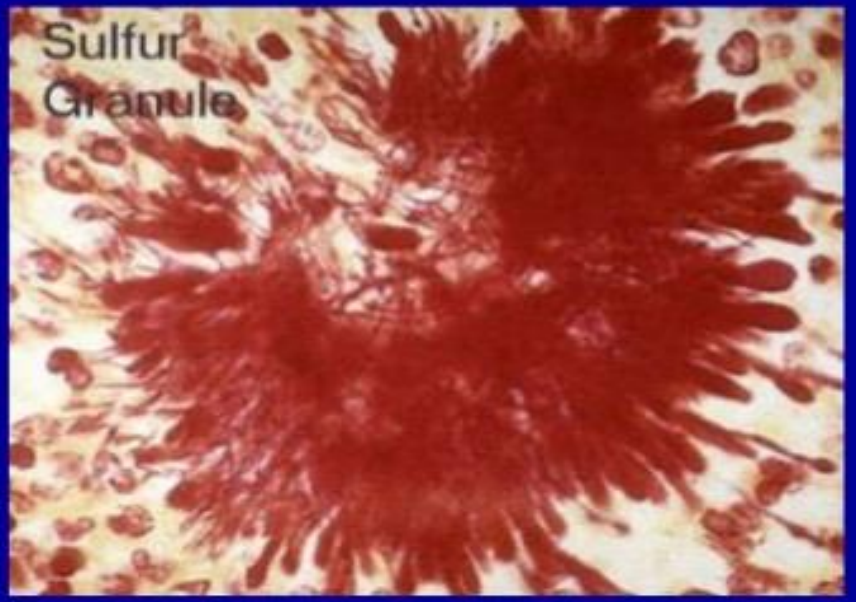
Actinomycosis – Lab Diagnosis

- Macroscopic examination of Granules
- Microscopy
  - Gram stain
- Isolation / Anaerobic Culture
- Serology – Not useful
- Molecular diagnostic tests - PCR

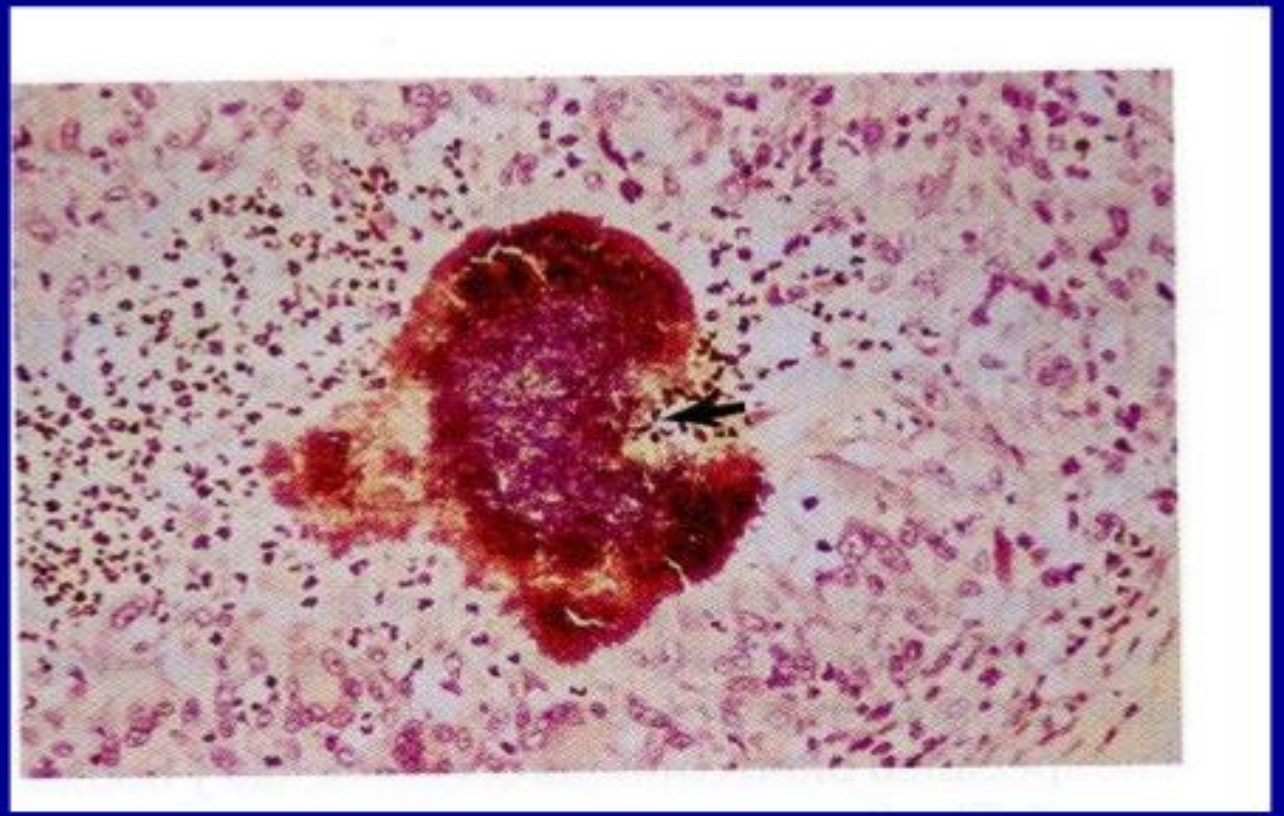
Macroscopic examination of Granules

- Yellow in colour (Hence the name Sulur granules)
- But may be white / brown
- Firm and round
- Size : 0.5 - 5mm in diameter

Sulfur granule



Granules



Treatment - Actinomycosis

- Penicillin

Aerobic Actinomycetes

Nocardia
Actinomadura
Streptomyces

Nocardia - Taxonomy

(Domain): Bacteria

(Kingdom): Actinomycetota

(Class): Actinomycetia

(Order): Mycobacteriales

(Family): Nocardiaceae

(Genus): Nocardia

(Species): ***N.asteroides, N.brasiliensis etc.***

Nocardia spp.

Three clinically important species

- *N. asteroides,*
- *N. brasilensis*
- *N. caviae*
  - Morphology and cultural characteristics
    - Gram positive branching filamentous bacteria
    - May fragment to bacillary or coccoid forms
    - Aerobic
    - The organisms are weakly acid fast or non acid fast
    - Ubiquitous in soil

Culture

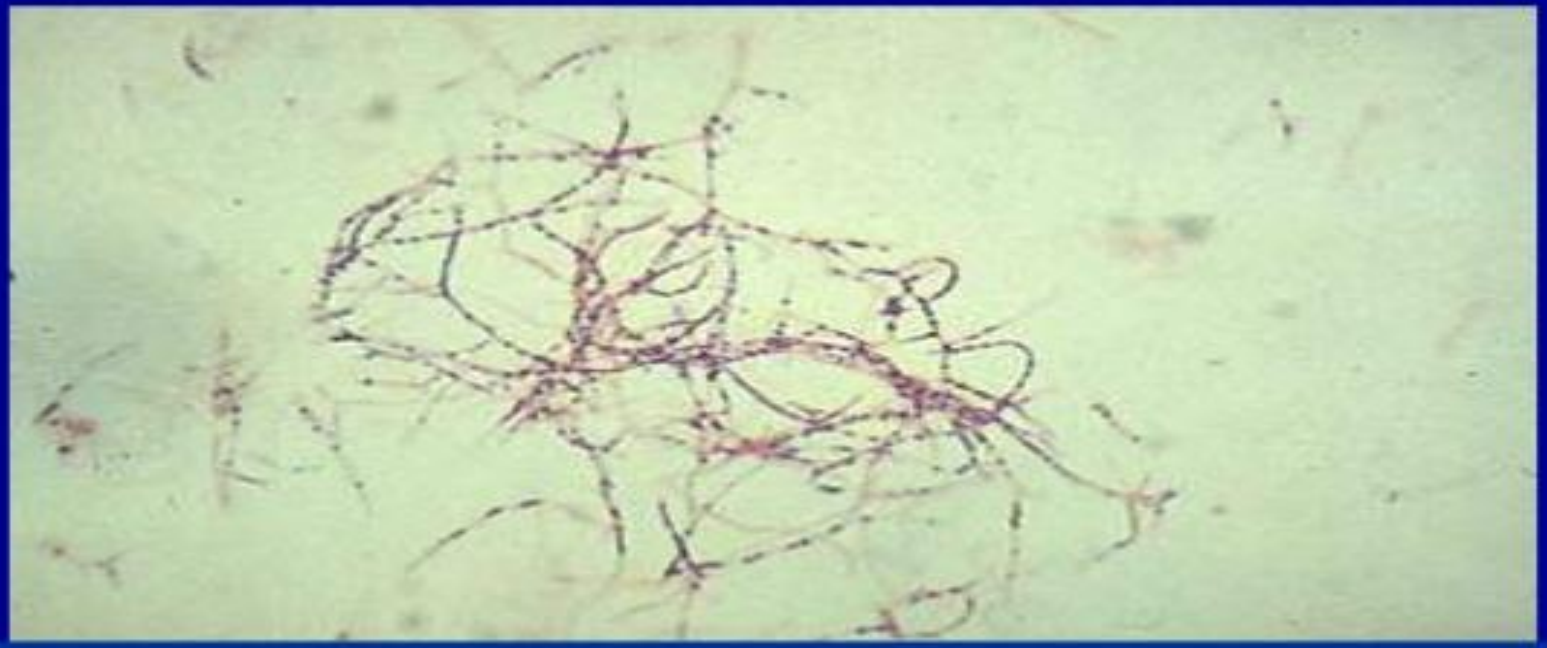
- Nocardiae grow on blood agar, although growth is better on enriched media including Löwenstein-Jensen medium, brainheart infusion agar and Sabouraud's dextrose agar containing chloramphenicol as a selective agent.
- Growth is visible after incubation for between 2 days and 1 month; selective growth is favoured by incubation at 45°C. Colonies are cream, orange or pink coloured; their surfaces may develop a dry, chalky appearance, and they adhere firmly to the medium
- On tap-water agar, *Nocardia* species have recursively branching hyphae with aerial hyphae.

Culture character

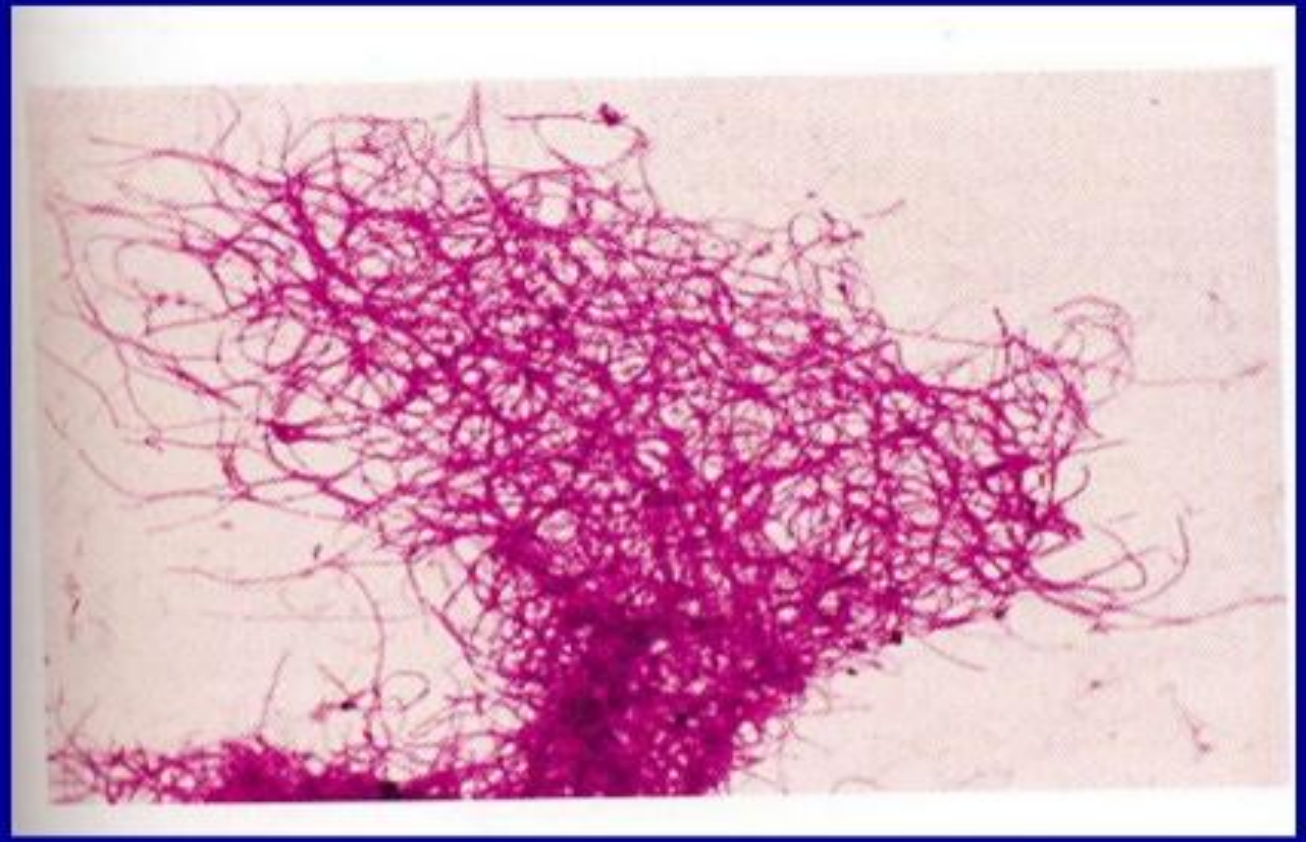
- Plate culture of the bacteria Nocardia asteroides grown on 7H10 agar plates at 37° C.
- Media: Nutrient agar, sabouraur agar, brain heart in fusion agar, yeast extract malt extract agar.
- Specimens with mixed flora can over grow the Nocardia colonies
- Selective media may increase yield:
  - **Thayer-Martin agar with antibiotics**
  - **paraffin agar.**
  - Buffered charcoal-yeast extract (BCYE) medium

Nocardia culture on Blood Agar

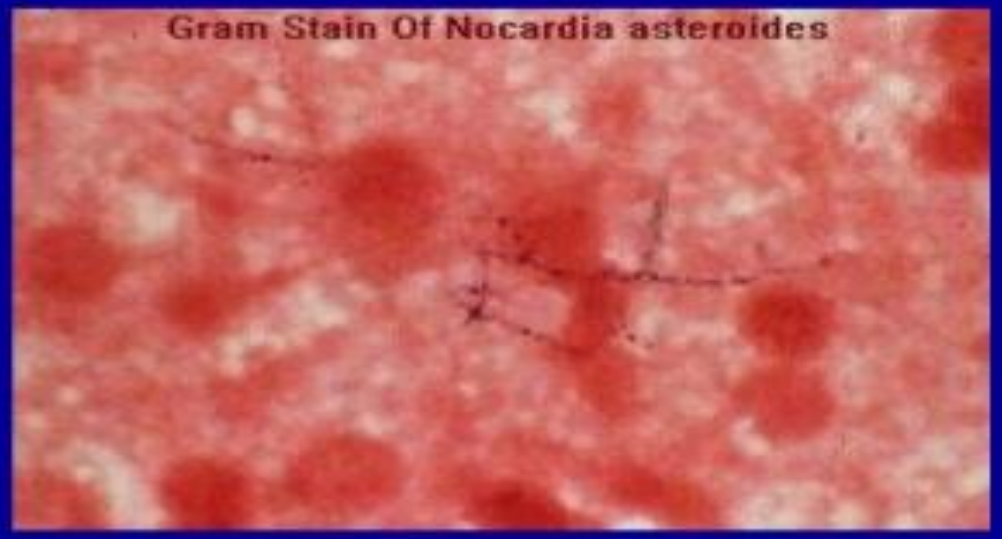
Nocardia – Gram stain



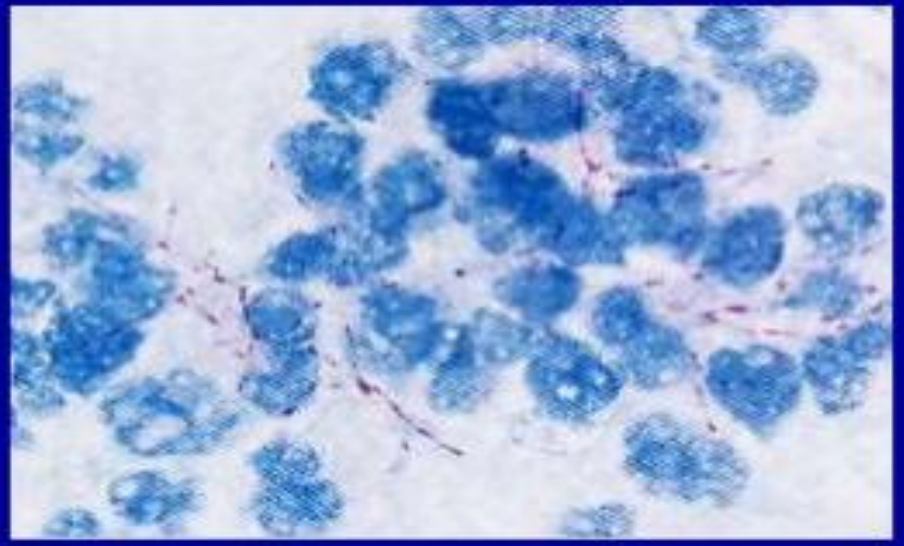
Nocardia acid fast stain



Nocardia spp.



Gram staining



Modified Acid Fast Staining

Biochemical test

- hydrolysis of casein, tyrosine, and/or xanthine, (2) presence of urease, (3) utilization of rhamnose, and (4) positive resistance to lysozyme.

Table 1: Hydrolysis Tests for differentiating *Nocardia* strain

	Casein hydrolysis	L-tyrosine hydrolysis	Xanthine hydrolysis
N. asteroides complex, N. farcinica, or N. nova	-	-	-
N. brasiliensis	+	+	-
N. otitidis	-	+	-
N. caviae	-	-	+
Streptomyces or Nocardiopsis	+	+	+

Spectrum of illness

- Skin / Soft tissue infections most common presentation
- Can spread hematogenously in rare cases
  - CNS & pulmonary System
- Persons with impaired host defense are more likely to develop systemic disease

Nocardiosis – Clinical manifestations

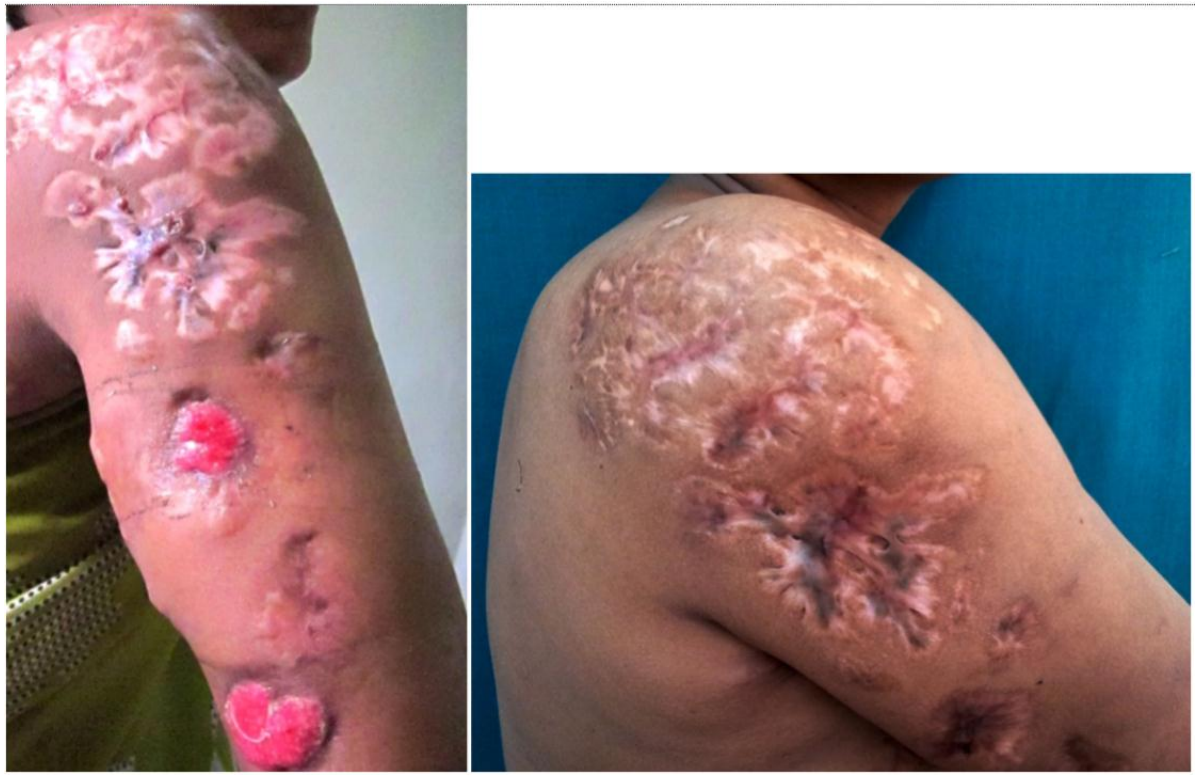
- Three broad types
  - Mycetoma
    - Most common cutaneous manifestation of *N. brasiliensis* worldwide
    - Chronic, indurated, granulomatous masses, mostly found on the Lower Extremities
    - Draining nodules & sinuses that contain sulfur granules
    - tend to invade underlying connective tissue, muscle, bone

Nocardiosis – Clinical manifestations

- Localized cutaneous Nocardiosis
  - Cellulitis, subcutaneous abscesses, pustules, pyoderma, ulcerations
- Pulmonary Nocardiosis
- Lymphocutaneous Nocardiosis
  - Also called the "sporotrichoid" form of nocardiosis
  - Rare

Nocardia asteroides

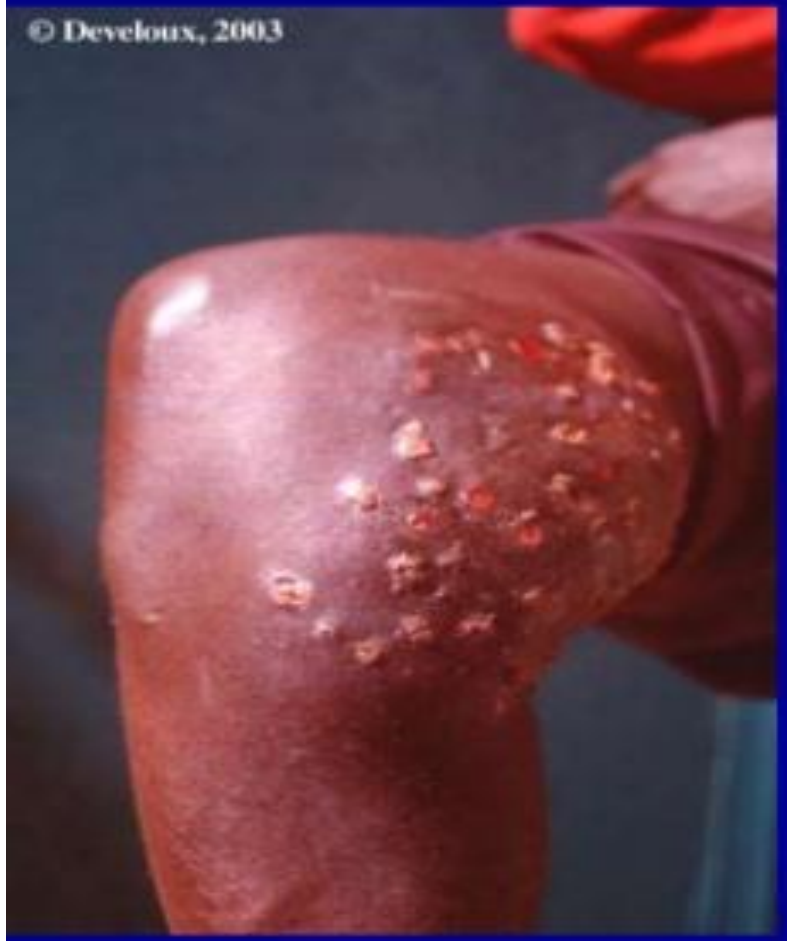
Nocardia brasiliensis

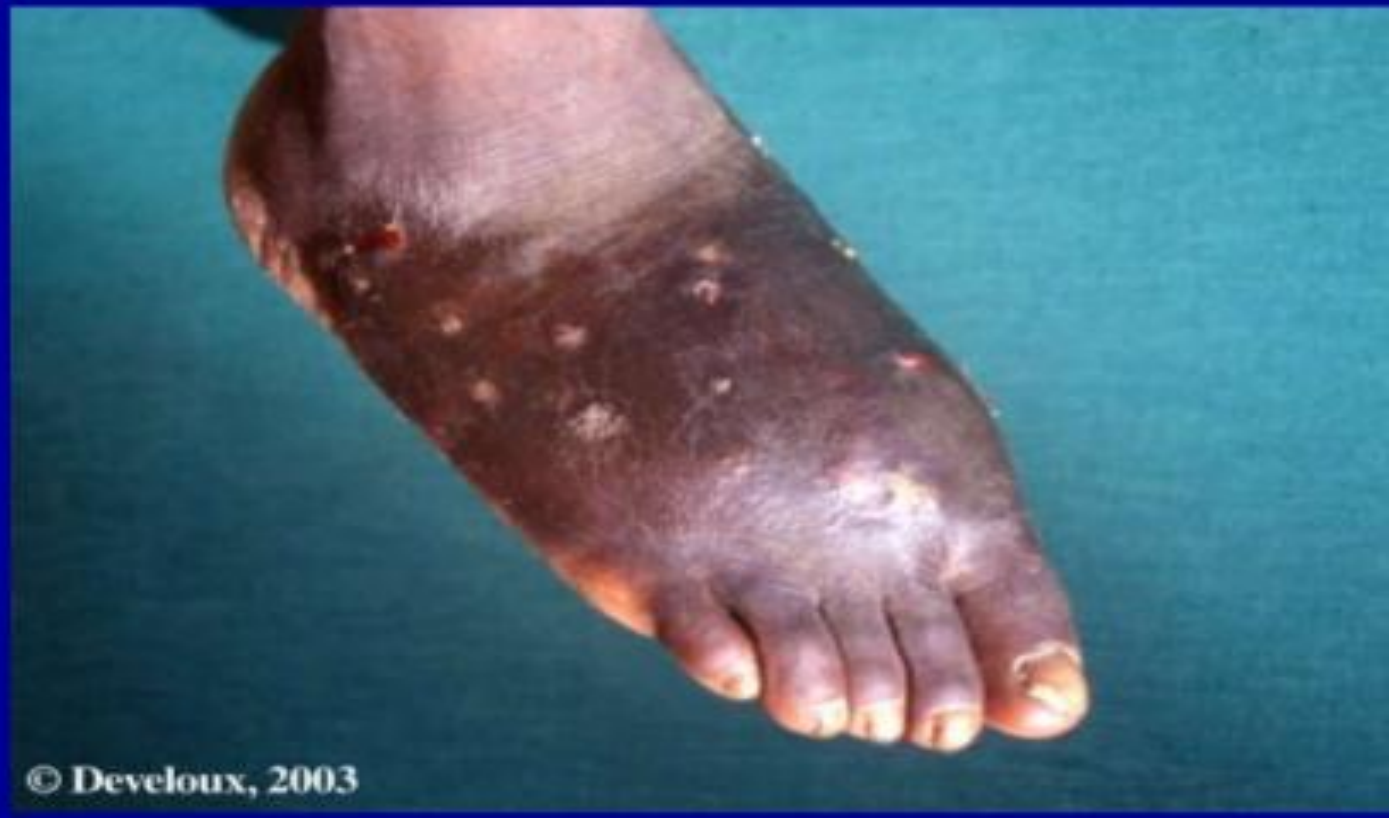


Mycetoma

- Organism enters the body through breaks in the skin
- Causes a localized infection involving skin, cutaneous, and subcutaneous tissue.
- The three most characteristic features seen in Mycetoma
  - » **swelling (Tumifaction)**
  - » **draining sinuses**
  - » **granules**
  - – This disease can also be caused by fungi

Mycetoma –Nocardia spp.





Nocardiosis

- Pulmonary nocardiosis
  - Localized or disseminated disease
  - Occurring after inhalation of organisms.
  - Pulmonary infections resemble tuberculosis
  - May disseminate to brain and meninges
  - Usually a disease of compromised hosts.

Laboratory Diagnosis

- Macroscopic examination of granules
- Direct Microscopy
  - Gram Staining
  - Ziehl Neelsen's staining
- Culture
  - Specimens should be inoculated onto
    - **7H10 agar**
    - **Lowenstein-Jensen agar**
    - **Brain heart infusion agar**
  - Orange, dry, crumbly, and adherent colonies
- Serology – Not useful
- Molecular Diagnosis - PCR

Nocardiosis - Treatment

- Mycetoma – aminoglycosides
- Pulmonary Nocardiosis – sulfonamides

Nocardiosis - Treatment

- Optimal duration of tretment not known
- Clinical outcome related to the duration of antibiotic therapy
- Tendency of Nocardia to recur
  - Treatment best continued for 3-12 months.
    - **depending on severity of disease**
    - **immune status of pt.**
  - Immunocompromised hosts, consider indefinite low-dose prophylaxis after full-dose therapy is completed

Other causes of actinomycotic mycetoma

- Actinomadhura madhurae
- Actinomadhura palletieri
- Streptomyces somaliensis