وَقُل رَّبِّ زِدْنِي عِلْمًا

النادي الطبي

# RESPIRATORY SYSTEM

## HAYAT BATCH

SUBJECT : Microbiology

LEC NO. : 4

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# Mycobacteria

They are called "mycobacteria" because they were originally thought to be fungi due to their similar appearance under the microscope. However, they are actually a type of bacteria, not fungi.

## Medically important Mycobacteria

- *M. tuberculosis
- *M. bovis

} Causative agents of tuberculosis in man

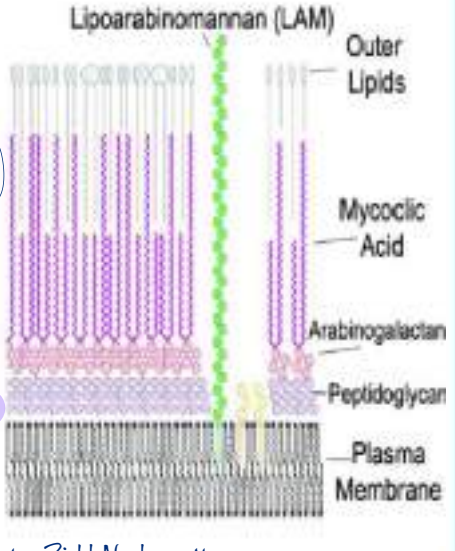
- *M. Leprae → Causative agent of leprosy
- *Atypical mycobacteria

## General characters of Mycobacteria:

- Slender rods (bacilli), non-spore forming, strictly aerobic.
- Difficult to stain with ordinary stains (e.g. Gram stain) because of a **high lipid** content **(mycolic acid)** (40-60%) in the cell wall.
- Stained with special stain **Ziehl-Neelsen (Z.N)** that depend on application of heat and concentrated dye.
- Once stained , they retain the stain and resist decolorization with acids, that is why described as "**acid fast bacilli** " **(AFB)**.

لهيك رح أصبغها ب ⬇️

❌ Gram positive/ Gram negative




ال Ziehl-Neelsen عبارة عن ثلاث خطوات رئيسية

الخطوة الأولى بستخدم primary stain لونها احمر تصبغ ال organism و خلفية الشريحة

الخطوة الثانية decolorization with acids حتى ال acid يشيل اللون من الشريحة

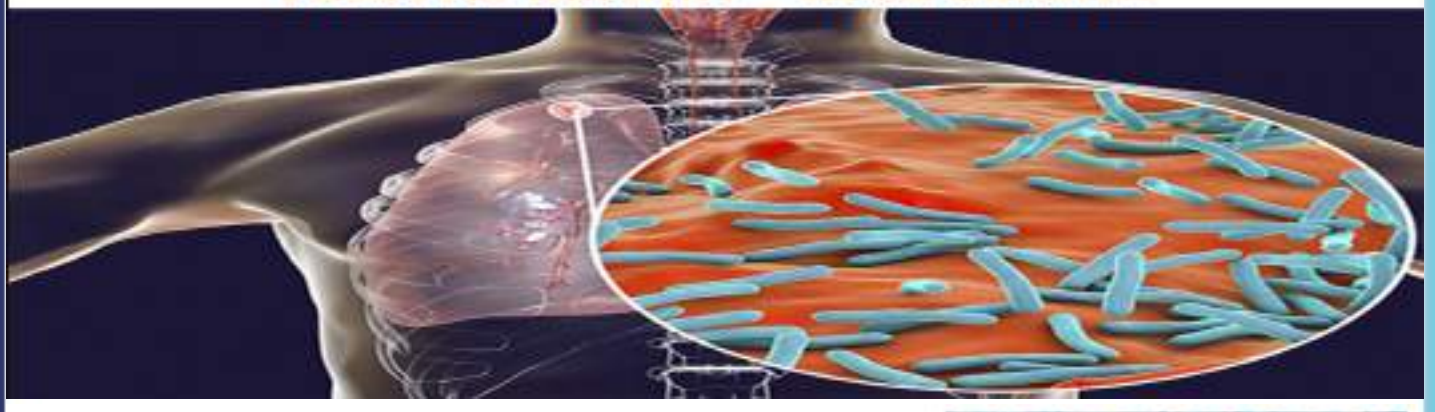
الخطوة الثالثة counterstain صبغة بكون لونها ازرق فهيك بكون استخدمت صبغتين مختلفات بألوان مختلفة

When the Ziehl-Neelsen stain is applied to a sample containing acid-fast bacteria, the carbol fuchsin penetrates the cell wall and stains the bacteria red. During the decolorization step with acid-alcohol, the acid-fast bacteria retain the stain due to the waxy layer in their cell wall, while other bacteria lose the stain and appear colorless. This property of retaining the stain despite exposure to acids is why acid-fast bacteria are described as "acid-fast bacilli"

# Respiratory System

## MYCOBACTERIUM TUBERCULOSIS

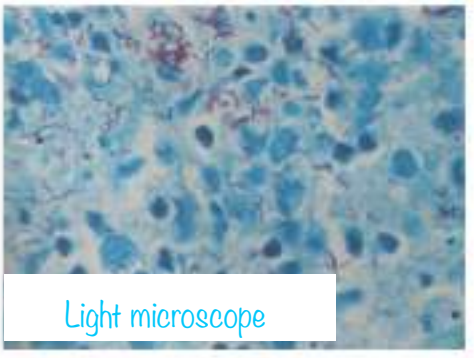
"Tubercle bacillus" "Koch bacillus"



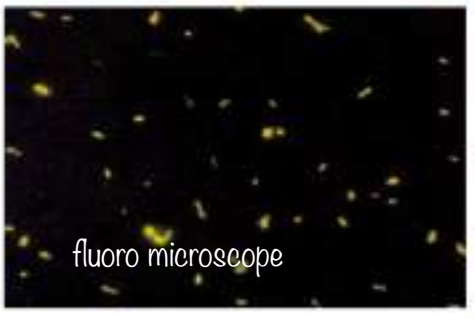
### Morphology:

- Thin straight or slightly curved **rods.** bacilli
- **Non motile**, **non-sporing** and **non-capsulated**.
- They stained by **Z.N (Hot)** or **Kinyoun (Cold)** بديل ال Z.N stain and appear as thin **pink rods** arranged singly or in small groups **in a contrasting blue background.**
- They can be stained by **fluorochrome** (fluorescent) stains (e.g. auramine, rodamine).

Organism = yellow
Background = black



Light microscope



fluoro microscope

### Cultural characters:

So, it's affecting the area with more oxygen in the body.

- They are **obligate aerobe** (upper lobe of the lung).
- They are **slow growers**, growth appears after 4-6 weeks (doubling time 18 hs in contrast to <1 hour in most bacteria).
- Types of media:

Selective media

1) Egg based media such as **Lowenstein-Jensen (L-J)** medium & Dorset's egg medium.

ال growth اسرع = ثلاث أسابيع

2) Agar based media e.g. Middlebrook's 7H10,7H11 agar.
3) Fluid media e.g., Middlebrook's 7H9.

**L-J medium**

# Respiratory System

## Resistance & Sensitivity:

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high lipid content in cell wall

*They are highly resistant to :

- Dryness (survives in dried sputum for long periods).
- Chemicals, many acids and alkalis.
- Antibiotics.

*They are killed by:

- Sunlight
- U.V. rays
- 5%phenol
- Heat (60°C for 20 min.) (**Pasteurization can kill them in milk**).

## Virulence Factors:

1. **High lipid of cell wall (Mycolic acids),** responsible for:

**Resistance to:** Antibiotics, acidic and alkaline compounds, Osmotic lysis via complement.

**2. Cord factor:** Virulent strains grow in a characteristic "Serpentine" cordlike pattern.


**3. Exported repetitive protein Erp & PKnG:** Main pathogenesis

Inhibit phago-lysosomal fusion.

The cord factor contributes to the integrity of the mycobacterial cell wall, which is important for resisting host immune defenses and antimicrobial agents.

The cord factor enhances the ability of M. tuberculosis to interact with and invade host cells

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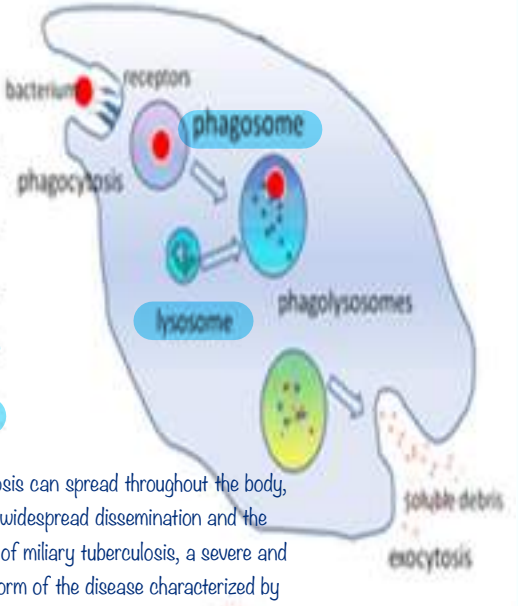
## Pathogenesis:

- Tubercle bacilli do not contain or produce toxins.
- Their pathogenicity depends upon the fact that the organism **survives and multiplies in macrophage** within a vacuole called a phagosome as it produces a specific protein that prevents phago-lysosomal fusion and so, escape the degradation by lysosomal enzymes.
- It is an intracellular organism.




M. tuberculosis can spread throughout the body, leading to widespread dissemination and the development of miliary tuberculosis, a severe and often fatal form of the disease characterized by the formation of numerous small tubercles in multiple organs.

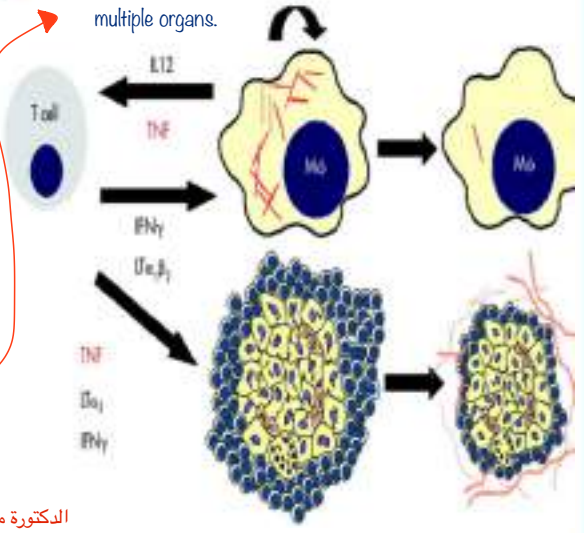
## Immunity Against Tuberculosis

- On primary infection, the patient develops:

1- **Cell mediated immunity (CMI) (Delayed-type = type IV hypersensitivity)** (Granuloma formation) that leads to localization of tubercle bacilli, retards their multiplication, limits their spread.

Patients deficient in cellular immunity, such as AIDS patients, are more susceptible to disseminated (miliary) tuberculosis.

2- Circulating antibodies forms but has little role.




الدكتورة ما ذكرت الشرح بالتفاصيل بس للفهم

1. Initial Infection: When M. tuberculosis enters the body, it is usually phagocytosed by macrophages, which are specialized immune cells tasked with engulfing and digesting pathogens. However, M. tuberculosis has developed mechanisms to survive and even replicate within macrophages, allowing it to evade the immune system's initial response.
2. Activation of Cell-Mediated Immunity (CMI): As the infection progresses, the body's adaptive immune response is activated, primarily through cell-mediated immunity (CMI). This involves the activation of T lymphocytes (specifically CD4+ and CD8+ T cells) by antigen-presenting cells, such as dendritic cells and macrophages, which present M. tuberculosis antigens to the T cells.
3. Formation of Granulomas: Granulomas are organized structures composed of immune cells, primarily macrophages and T cells, surrounded by a cuff of fibroblasts and connective tissue. The formation of granulomas is initiated by the aggregation of activated macrophages and T cells at the site of infection.
4. Recruitment of Immune Cells: Chemokines and cytokines released by activated immune cells attract additional immune cells to the site of infection. This includes monocytes, neutrophils, and other leukocytes, which further contribute to the formation and maintenance of the granuloma.
5. Central Core Formation: Within the granuloma, infected macrophages containing M. tuberculosis undergo fusion to form multinucleated giant cells. These giant cells attempt to contain the infection by walling off the bacteria within the granuloma's central core.
6. Encapsulation: Fibroblasts and connective tissue surround the granuloma, forming a barrier that helps contain the infection and prevent its spread to surrounding tissues.

Overall, granuloma formation is a critical aspect of the immune response to M. tuberculosis infection, playing a central role in containing the pathogen and preventing its dissemination,

# Respiratory System

## Human Tuberculosis (TB)

- Caused mainly by the **human** and **bovine** types.
- **Human** type is transmitted airborne by **inhalation** of respiratory aerosol (droplet nuclei <5µm) which expelled from active TB patient when cough, speak, sneeze,.. These nuclei remain suspended in air for several hours. Its initial site of infection is the **lung**.
- **Bovine** type is transmitted mainly by **ingestion** of unpasteurized milk of infected cattle **(zoonosis)** and its initial site of infection is the **intestine**.

لما ال organism يدخل الجسم لأول مرة

### Primary pulmonary tuberculosis:

- Characterized by a small lesion called **"Primary complex"** which consists of:
  - * Ghon focus (T.B. granuloma) in the lung (mid-zone).
  - * Lymphangitis and lymphadenitis of the draining lymph nodes.

- The T.B granuloma become surrounded by fibrous tissue (Tubercle), undergone central caseation necrosis (cheese like).
- **Fate of primary lesion:**

- In most cases, it is asymptomatic and tubercles heal by fibrosis and calcification leaving the person immune and hypersensitive **(tuberculin positive)**.
- Small foci containing **dormant viable** organisms **(Simon foci)** may be formed and often become sites of reactivation **(Latent TB)**
- Only small % (immunocompromised) progress into active or disseminated T.B.

They can persist in the body for years without causing symptoms, a condition known as latent tuberculosis (Latent TB).

Disseminated tuberculosis occurs when the infection spreads beyond the lungs to other organs, such as the kidneys, bones, or brain.

Reactivation: In some cases, particularly when the immune system weakens or becomes compromised, such as due to other illnesses or medications, the dormant bacteria in the Simon foci can reactivate. This reactivation leads to the development of active tuberculosis, where the bacteria multiply and cause symptoms.

# Respiratory System

## Secondary pulmonary tuberculosis:

- Is the most common form of **clinical** tuberculosis.
- It may be: **reactivation** of old primary lesion or **reinfection.**
- Occurs mainly in **immunocompromised,** debilitated or diabetic patients.
- **Spread of the organism occurs by two mechanisms:**

1) Local spread: -To other parts of the lungs (upper lobe), OR (More oxygenated)

-A tubercle **cavitate,** erode a bronchus, empty its contents, and spread the organism to other persons if expectorated **(Open TB).**

2) Hematogenous spread: which result in **miliary T.B.** the bacteria can spread beyond the lungs through the bloodstream. Miliary tuberculosis can affect multiple organ systems and is associated with a more severe and disseminated form of the disease.

**Cavitation** tubercles can enlarge and coalesce, forming larger cavities within the lung tissue.

**Bronchial Erosion** As these cavities grow, they can come into contact with nearby structures, including the bronchial tubes

**Emptying Contents** If a cavity erodes into a bronchial tube, its contents, including bacteria, dead cells, and other debris, can be expelled from the lungs when the infected person coughs, sneezes, or even talks.

**Spread to Others (Open TB)** the infected person is now actively expelling infectious material into the air. If another person inhales these infectious droplets, they can become infected with tuberculosis.

## Symptoms of active TB disease:


Cough lasting 3+ weeks


Coughing up blood or sputum *(phlegm from deep inside the lungs)*


Chest pain


Weakness or fatigue


No appetite


Weight loss


Fever and/or chills


Night sweats

**Note that the sputum is yellowish green or may be coughing blood (hemoptysis).**

## Laboratory Diagnosis

**Specimens:** Sputum (3 consecutive days) or broncho-alveolar lavage.

**1- Direct microscopic examination:**

***Z.N stain & Kinyoun:** low sensitivity (Require large number of bacilli).

-Positive film is highly suggestive, **negative film does not exclude T.B**.

***Flourochrome stain:** More sensitive and allow more rapid screening than Z.N.

**2- Culture:**

-Culture is the **gold standard and the most conclusive** method.

-L.J medium **(up to 8 weeks)** or more rapid Middlebrook 7H9 **(~3 weeks)**.

**3- Polymerase Chain Reaction (PCR):** Rapid & sensitive.

## Tuberculin Test "Mantoux test"

**Principle:** It is skin allergic test used to detect **cell mediated immunity** to tubercle bacilli which become detectable **few weeks** after natural infection or BCG vaccine.

متى ال immune system بكون cell mediated immunity ؟؟

بدها 3 أسابيع حتى تتكون و
لما تتكون بكون الشخص عنده
القدرة يتعامل مع tb

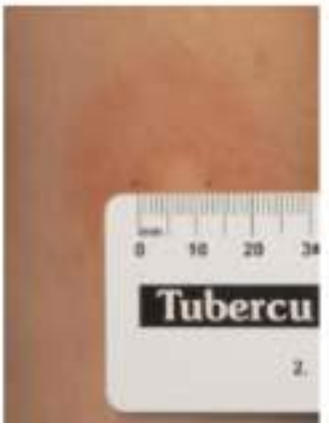
**Procedure:**

Intradermal injection of 0.1ml of **PPD (Purified Protein Derivative).**

Read the test **48-72 hours.**

Measure the diameter of the induration using mm ruler.

"**Only the induration**", which is localized hard papule, is measured, even if there is surrounding erythema).

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# Interpretation of Tuberculin test

| An induration of 5 or more mm | An induration of 10 or more mm | An induration of 15 or more mm |
|---|---|---|
| Considered positive for:<br>1. People with previous history of TB.<br>2. Close contacts of TB patients.<br>3. People with HIV infection.<br>+organs transplant | Considered positive for:<br>1. People in endemic areas where TB is common.<br>2. Healthcare workers.<br>3. People with certain medical conditions such as diabetes.<br>4. Unvaccinated children younger than 4 years old. | considered positive even in absence of any risk factor for TB. |

**Positive Tuberculin dose not differentiate between active or latent T.B**

## ➢ Negative Test:

A negative test means that there is no infection at all or a very old healed one.

**Tuberculin is a good negative test.**

## ➢ False Negative Test:

**1. Anergy:** is the inability to react because of a weakened immune system, e.g. Severe T.B, HIV infection, Some viral infections or cancer.

**2. Recent T.B:** it takes 2-10 weeks for tuberculin test to become positive.

## ➢ False Positive Test:

1-Infection with other non-tuberculous mycobacteria.

2- BCG vaccine (The test reactivity induced by vaccine wanes with time).

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# Treatment:

- **First line anti-tuberculous drugs:** more effective with less side effects.

Isoniazid (INH), Rifampicin, Pyrazinamide, Ethambutol.

- **Second line anti-tuberculous drugs:** less effective with more side effects.

Fluorquinolones, Streptomycin, Amikacin, …

- Second line drugs can be used in patients whose infecting strains are resistant to the first line drugs.

## Treatment of TB should be:

### 1-Long Duration:

Response of tuberculosis to treatment is slow, this is due to the facts that:

- Intracellular location of the organisms.
- Caseous material interferes with penetration of the drugs.
- The slow growth of the organism.
- Metabolically inactive "persisters" within the lesion in chronic cases which may not be eradicated easily by antit-uberculous drugs (source of reactivation in the future).

### 2- In Combination: 2-4 drugs simultaneously to: Synergistic Effects

- Reduce development of resistance.
- Reduce toxicity of the drugs.

This is because the impact of each drug's side effects is diluted when combined with other drugs. So, even if one drug in the combination has side effects, they might not be as severe because they are balanced out by the other drugs in the regimen.
Additionally, some drugs may actually counteract the side effects of others. For instance, if one drug causes gastrointestinal disturbances, another drug in the combination might have a protective effect on the stomach lining, reducing the severity of those disturbances.

# Resistant mutants Worldwide problem

- **Multidrug resistant TB (MDR-TB):** means tubercle bacilli resistant to both isoniazid (INH) and rifampicin.

- **Extensively (Extremely) drug resistant TB (XDR-TB):** It is defined as MDR + resistance to fluorquinolones and at least one second-line injectable drugs. Results from inadequate treatment of MDR-TB.

Second line

- Because drug resistance is a problem, antibiotic sensitivity testing should be performed for all isolated organisms.

## Prevention:

### Vaccination: BCG "Bacillus of Calmette-Guérin" vaccine:

- This is a **living attenuated** vaccine prepared from a **bovine** strain.
- It is given as a **single dose** of 0.1 ml by intradermal injection in the left deltoid region.
- It is given to all children during the first month of life.
- It is also given to adults exposed to infection e.g. nurses, doctors and contacts of the case.
- It should **NOT** be given to immunocompromised people.
- It loses its effectiveness over time, usually within 5 to 15 years

# ATYPICAL MYCOBACTERIA

**Non-tuberculous mycobacteria "NTM"**
**Mycobacteria other than tuberculosis "MOTT"**

- They normally found in soil and water.
- Transmission is from the environment. **NO** person to person transmission.
- They are of **low pathogenicity** for man but occasionally they cause **opportunistic** infections especially in **immunocompromised** persons.
- They cause pulmonary diseases which are **indistinguishable** clinically, radiologically and histologically from that caused by the human tubercle bacilli, but tend to be **more chronic and difficult to be eradicated.**
- e.g. **M. Avium Complex (MAC)** (M. avium, M. intracellulare, M. chimera).

1. Clinical Presentation: The symptoms of pulmonary diseases caused by atypical mycobacteria can closely resemble those of tuberculosis. These symptoms may include cough, fever, night sweats, weight loss, fatigue, and shortness of breath. Since these symptoms overlap with those of TB, it can be challenging to differentiate between the two based solely on clinical presentation.
2. Radiological Findings: Both TB and diseases caused by atypical mycobacteria can produce similar radiological abnormalities in the lungs. These abnormalities may include nodules, cavities, infiltrates, and fibrosis. As a result, the radiological features observed on chest X-rays or CT scans of patients with NTM infections can be difficult to distinguish from those seen in TB cases.
3. Histological Features: Histological examination of lung tissue samples from patients with NTM infections may reveal granulomas, which are also characteristic of tuberculosis. Granulomas are clusters of immune cells that form in response to chronic inflammation caused by the mycobacterial infection. Additionally, other histological features such as caseating necrosis may be present in both NTM and TB infections, further complicating the differentiation between the two.

# Respiratory System

1) A 56-year-old Indian woman with a chronic cough, night sweats, and weight loss for 4 months goes to a pharmacy for a medication. In addition to a cough suppressant, the medicine she can buy over-the-counter in her country contains isoniazid. Her symptoms initially improve dramatically, but return in a few weeks and her illness continues to progress. What is the most likely diagnosis?
(A) Asthmatic reaction to the medication
(B) Chronic bronchitis
(C) Mycoplasma pneumonia
(D) Pneumococcal pneumonia
(E) Tuberculosis

The answer is E: Tuberculosis..

2) Which one of the following is characteristic of mycobacteria?
A. They contain mycolic acids.
B. They are resistant to inactivation by heat.
C. They grow extracellularly.
D. They are anaerobic.
E. They are spore forming.

Correct choice
A. Mycobacteria are unique in that their cell walls contain high concentrations of mycolic acids. Mycobacteria are not particularly heat resistant, as witnessed by their susceptibility to pasteurization. They are aerobic, intracellular organisms that do not form spores.

3) The treatment of tuberculosis
A. is initiated with a single "first-line" drug.
B. is initiated after the results of sensitivity testing is available.
C. is most effective in patients with chronic or arrested tubercles.
D. may last 2 to 3 weeks.
E. should be directly observed whenever possible.

Correct answer = E. Where directly observed therapy used, the incidence of new cases falls dramatiacally and success of therapy is much more likely. The standard procedure is to begin treatment with two or more drugs to prevent emergence of resistant strains. Sensitivity tests are an important guide to modifying treatment, but sensitivity data are not required to initiate therapy. In chronic or arrested tubercles, the organisms are nonproliferating, and therefore are not susceptible to many antimicrobial agents.

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## *Mycobacterium tuberculosis*

### Pathogenesis/Clinical Significance

*M. tuberculosis* survives and grows in host macrophages, where it can remain viable but quiescent for decades. Immunosuppression can lead to reactivation. *M. tuberculosis* produces no demonstrable endo- or exotoxins.

Tuberculosis is the principal chronic bacterial disease in humans, and is the leading cause of death from infection worldwide. Transmission is by aerosol droplets produced by coughing, and depends on crowded conditions and poor ventilation.

- **Tuberculosis:**

  Tubercles (productive granulomatous lesions) form in the lung following infection by *M. tuberculosis*. Their formation is mediated by the host immune response. The lesion may arrest and become fibrotic and calcified, or it can break down, resulting in spread of the infection via the lymph and bloodstream. *M. tuberculosis* can seed different tissues, causing, for example, chronic pneumonitis, tuberculous osteomyelitis, or tuberculous meningitis. If active tubercles develop throughout the body, this serious condition is known as miliary (disseminated) tuberculosis.

### Treatment and Prevention

- **Treatment:** A long course of combined antibiotic treatment (6 months or more) with isoniazid, rifampin, pyrazinamide, and ethambutol is required for a cure.
- **Prevention:** Bacille Calmette-Guerin vaccine is available, and is used for tuberculin-negative individuals under sustained heavy risk of infection. Isoniazid is used prophylactically, for example, for tuberculin-positive, asymptomatic individuals, who need immunosuppressive therapy for other illnesses.

### Laboratory Identification

- Acid-fast bacteria can be observed in clinical specimens treated with Ziehl-Neelsen stain.
- Nucleic acid probes can be used to detect *M. tuberculosis* DNA that has been amplified by polymerase chain reaction.
- The organism can be cultured on specialized media such as Lowenstein-Jensen agar.

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# Bacteria – Mycobacteria


Mycobacterium Leprae - The good, the bad, and the lion faced

1. Thrives in cool temperatures leading to growth in extremities
2. Acid Fast Gun slinger - Acid fast, carbol fuscion stain
3. Tassels on coat and jacket - Mycolic acids
4. Armadillo County - Armadillos are the major reservoir - commonly called Hanson's disease
5. Hanson the Armadillo – Hanson's Disease
6. Clinical presentations
    a. Tuberculoid # 1
        i. Jail Cell 1 - Helper TH1 cells stimulate macrophages to engulf the bacteria in cell mediated immunity
        ii. Well demarcated bald spot - Mild symptoms, well demarcated hairless lesions on skin
        iii. Shovel in mound of dirt - Lepermans skin test - test for immune reaction, similar to TB Test, wheel will form if positive
    b. Lepromatous presentation #2
        i. Jail Cell 2 with laughing prisoner- TH2 cells promote humoral (humorous) response
        ii. Prisoner breaking out of cage - Bacteria being unable to contained in macrophages
        iii. Touching each other - High chance of transmission human to human
        iv. Prisoner are wearing glove and stockings - Distal portions are affected in a glove and stocking pattern
        v. Extensor surfaces w/ patches - Numerous extensor surfaces are cooler and present with disease of demarcated lesions
        vi. Mask on the gunslinger - Leonine faces, facial deformity.
7. Treatment - multi drug therapy for long time
8. Deputy carrying a rifle - TH1 Dapsone and rifampin for 6 months (deputy and rifle)
9. Deputy with rifle and white cloth, and cloth escape rope - TH2 Dapsone, rifampfin, and Clofazimine for 2-5 years