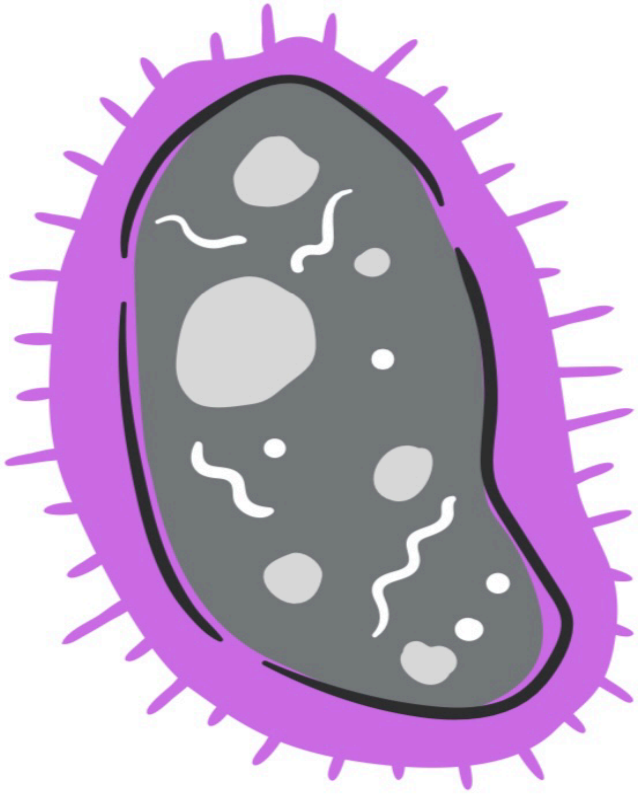
GIS

Sheet no.6

Microbiology



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أي شيء تحته خط ذُكرَ بالسلايدات لكن لم يذكر بالمحاضرة + ركز على ماهو غامق او لونه مَختلف عن سياق النص

*The Brucellae, Leptospira and Mycobacterium of the GIT:

In this lecture we will continue talking about bacterial infections of the gastrointestinal tract, particularly about the following:

1- Brucella: the causative agent of brucellosis

2- Leptospira interrogans: the causative agent of leptospirosis. 3- Mycobacterial tuberculosis: mainly abdominal tuberculosis.

Now let's get into details :

Brucella :

- Brucellae genus has multiple species (some DNA related studies have shown that there is actually only one member in the genus, B. melitensis, with multiple biovars (reservoirs))
  1- **Brucella melitensis** (the most common cause of human Brucellosis) and the reservoir is sheep and goats.
  2- **Brucella canis** and the reservoir is dogs.
  3- **Brucella abortus** and the reservoir is cattle (it is named abortus because it causes abortion in cattle but not in humans)
  4- **Brucella suis** and the reservoir is swine.

⇒ *Note*: other "species" of Brucella are only found in animals.

- Although each species of Brucella has a preferred host, all can infect a wide range of animals, including humans.

• Brucellae cause the human disease brucellosis (undulant fever(الحمى المتموجةor Malta fever or Mediterranean fever) and is characterized by an acute bacteremic phase followed by a chronic stage that may extend over many years and may involve many tissues.

- Undulant fever : because it highly rises and decreases especially in afternoon time .

- ***Morphology and Identification :***
- They are gram negative but often stain irregularly, unencapsulated, aerobic, nonmotile, non-spore forming and are adapted to an intracellular habitat.
- The appearance in young cultures varies from cocci to rods 1.2 µm in length, with short coccobacillary forms predominating.
  Their nutritional requirements are complex.

*some of brucella species are microaerophilic like B.abortus

* They do not produce neither acid nor gas in sufficient amounts for classification. Do not ferment any carbohydrates (They are relatively inactive metabolically)

Catalase and oxidase positive (the 4 species that infect humans)
But urease negative

▪ ***Epidemiology:*** Brucellosis is a zoonotic (animal) disease with the main reservoir being wild and domestic animals. Humans become accidentally infected after being exposed to Brucellae species found in animals or their products.human to human transmission is rare .

⇒ The common sources of infection for humans are: 1- Unpasteurized milk and milk products like cheese (most commonly cheese made from unpasteurized goat milk)
2- Occupational contact (e.g. farmers, veterinarians, laboratory workers and slaughterhouse/abattoir workers) with infected animals, their feces, urine or tissues.

- Brucellosis may be acquired by **ingestion** (the most common route of transmission)(via GIT), **inhalation**, **mucosal** (via droplets) or **percutaneous exposure**. Percutaneous exposure includes needle sticks, abrasions or skin cuts which allow the entry of Brucellae.

- Due to close contacts with animals, Brucellosis used to be very endemic in our region which is why it is commonly known as Mediterranean fever. It was very common to the point that anyone who presents with undulant fever (temperature keeps going high and low) and an abnormal ***gait*** (walking pattern) was diagnosed with brucellosis until proven otherwise.

  • In general, microorganisms are classified into 4 biosafety levels (1 is the simplest and least pathogenic to humans while 4 is the severest). Brucellae is considered as biosafety level 3 which means you can't undergo studies with it in a standard laboratory unless using biosafety level 3 cabinet
  • B. melitensis and B. suis have historically been developed as biological weapons by several countries and could be exploited for bioterrorism.
  • Accidental injection of the live vaccine strains of B. abortus (S19 and RB51) and B. melitensis (Rev 1) can cause disease.

  ▪ ***Pathogenesis:*** The Brucellae are obligate parasites of animals and humans and are characteristically located intracellularly.

  - Despite being Gram-negative bacteria, Brucellae endotoxin activity is less severe than other gram-negative bacteria. They do not produce any exotoxin either.
    • Brucellae targets the **reticuloendothelial system** starting from macrophages and lymph nodes reaching the **liver, spleen and bone marrow.**
    • From the portal of entry, Brucellae pass the epithelial barrier and innate immune cells reaching the macrophages (antigen-presenting cells)→They multiply inside the macrophages and they get carried by these infected cells to parenchymatous organs especially parts of reticuloendothelial system (spleen, liver, bone marrow)

- ⇒ the traveling of the bacteria occurs via lymphatics and blood stream

• The Hallmark of brucellae pathology is the formation of ***granulomas*** in lymphatic tissue, liver, spleen, bone marrow, and other parts of the reticuloendothelial system. These granulomatous nodules consist of **epithelioid and giant cells, with central necrosis** and at an advanced stage peripheral fibrosis. In such lesions, the Brucellae are principally intracellular.

• The main histologic reaction in brucellosis consists of proliferation of mononuclear cells, exudation of fibrin, coagulation necrosis, and fibrosis.

▪ *Clinical Findings :*

❖ This disease can be **acute** after incubation period in between 1-4 weeks . The onset is insidious, with malaise, fever, weakness, aches, night sweats and musculoskeletal symptoms., and can be **chronic** characterized by weakness, aches and pains, low-grade fever, nervousness, and other nonspecific manifestations compatible with ***psychoneurotic symptoms.(depression in older people)***

❖ ***Possible presentations of brucellosis :***

1- Acute presentation with flu like illness , fever , malasia and , systemic manifestations

2- For younger people→fever + monoarthritis (inflammation of one of the joints) usually of the hip or knee joint. This is what affects the gait.

3- For older people→fever + low back pain because of the involvement of the vertebral column.

❖ There may be gastrointestinal and nervous symptoms, **lymph nodes enlargement, palpable spleen,** meningitis, cholecystitis, hepatitis with jaundice.

• Deep pain and disturbances of motion, particularly in vertebral bodies, suggest **osteomyelitis.** These symptoms of generalized Brucella infection generally subside in weeks or months, although localized lesions and symptoms may continue.

▪ *Diagnostic Laboratory Tests :*

➢ **A. Specimens**

Blood or biopsy material (lymph nodes, bone and bone marrow, spleen and so on) should be taken for culture, and serum for serologic tests.

➢ **B. Culture**

Brucella agar is used; a selective agar plate→then incubate in microaerophilic conditions. The medium is highly enriched and —in reduced form— is used primarily in cultures for anaerobic bacteria. Brucella species bacteria grow on commonly used media, including

trypticase-soy medium with or without 5% sheep blood, brain–heart infusion medium, and chocolate agar.

Note: The typical virulent organism forms a smooth and transparent colony upon culture

• Problems with Brucellae culture that may make serological tests preferable (next page) - The bacteria are hard to isolate due to their intracellular characteristics
The bacteria take a long time to grow needing an incubation period of at least 8 weeks.

- **C. Serology**
  - IgM antibody levels start rising in first week of acute illness and peak at 3 months.
  - IgG and IgA antibody levels rise about 3 weeks after onset of acute disease and peak at 6- 8 weeks, and remain high during chronic disease. (peak earlier during the infection)

*The serology criteria should include agglutination and non-agglutination tests:

1-Serum agglutination test : IgG agglutinin tiger above 1:80 or a four-fold rise in titer on a repeat specimen is presumptive diagnosis (indication for active infection). Note: usually when we test for acute infection, we look for IgM antibodies, but with Brucellae infection we look for IgG antibodies as they rise and peak earlier.

2-non- agglutination tests : ELISA to quantify IgG , usually above certain level it is considered positive.

- **Blocking antibodies** : it is a phenomenon occurs in brucellosis serum especially in agglutination tests when we take serum from patient and mix it with antigens were preserved previously in lab we will notice this phenomenon in order to overcome it we add antihuman globulins

- ***Treatment and immunity :***
  • Brucellae may be susceptible to tetracyclines, rifampin(1 g/day , doxycycline 100mg/ day), trimethoprim– sulfamethoxazole, aminoglycosides, and some quinolones. Symptomatic relief may occur within a few days after treatment with these drugs. However, because of their intracellular location, the organisms are not readily eradicated completely from the host.
- For best results treatment must be prolonged.combined treatment with a tetracycline (eg, doxycycline) and either streptomycin for 2–3 weeks or rifampin for 6 weeks is recommended.Type equation here.

- ***Prevention and control :*** well screening for the brucellosis in animals , once the infected animal get pregnant they start to secrete **erythritol** hormone which acts as growth factor for brucella and invites brucella from reticulendothilium systems to placenta to accumulate there , they causes placentitis end up with abortion of embryo , luckily this hormone does not found in humans .

• Eradication of brucellosis in cattle can be attempted by test and slaughter, active immunization of heifers with avirulent live strain 19, or combined testing, segregation, and immunization. Cattle are examined by means of agglutination tests.

• Active immunization of humans against Brucella infection is experimental.

• Control rests on limitation of spread and possible eradication of animal infection, pasteurization of milk and milk products, and reduction of occupational hazards wherever possible.

Leptospira

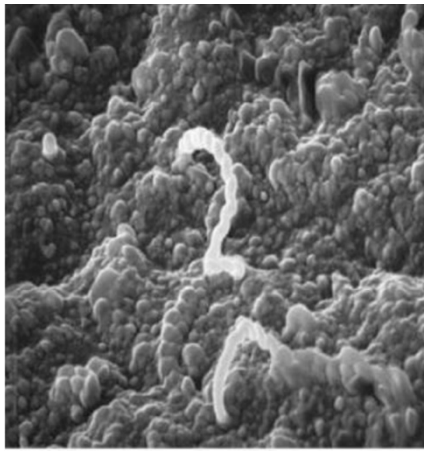
Pathogenic one

The genus Leptospira comprises two species: the pathogenic spirochete L. interrogans and the free-living saprophytic spirochete L. biflexa, now designated L. interrogans sensu lato and L. biflexa sensu lato, respectively.

• Leptospirae are tightly coiled, thin, flexible spirochetes (spirally shaped) 5–15 µm long, with very fine spirals 0.1–0.2 µm wide; one end is often bent, forming a hook.

• They are actively motile, which is best seen using a dark-field microscope.

• The bacteria can also be seen using transmission electron microscope as in this image. It's spiral with two periplasmic flagellae making it look like a question mark symbol.



• Leptospirae derive energy from oxidation of long-chain fatty acids and cannot use amino acids or carbohydrates as major energy sources. Ammonium salts are a main source of nitrogen.

• Leptospirae can survive for weeks in water, particularly at alkaline pH.

• The disease caused by pathogenic Leptospira species is called **leptospirosis. Or mud fever**

- ***Epidemiology:***

Leptospirosis is mainly a **zoonotic disease** but can also **affect human beings accidentally**

. • Rodents like mice and rats are the main animal reservoirs. Kidney involvement in many animal species is chronic and results in **the shedding of large numbers of leptospirae in the urine; which is probably the main source of environmental contamination resulting in infection of humans**. Human urine may also contain these spirochetes in the second and third weeks of disease

• Leptospirosis has a worldwide distribution but occurs most commonly in the tropics and subtropics because the climate and occasionally poor hygienic conditions favor the pathogen's survival and distribution.

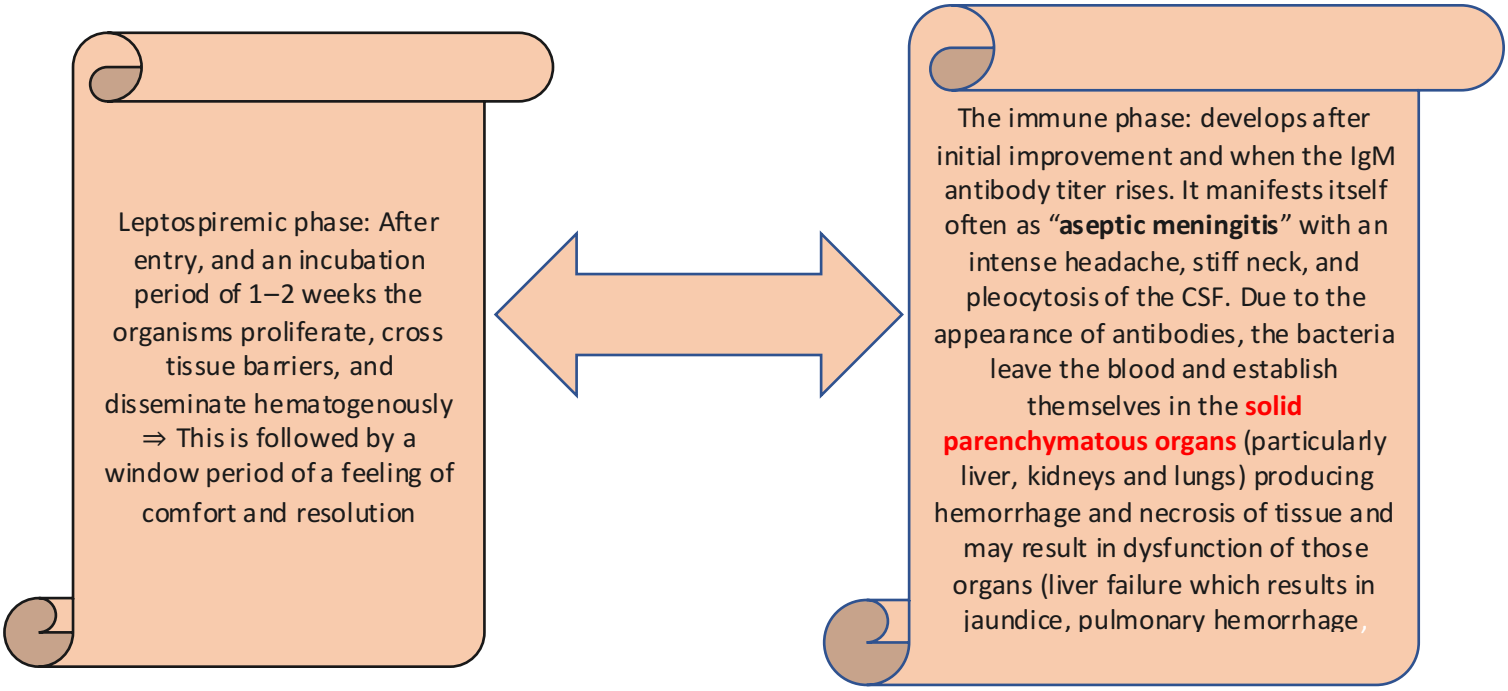
• Current information on global human leptospirosis varies but indicates that approximately 1 million severe cases occur per year, with a mean case–fatality rate of nearly 10%.

- ***Pathogenesis :***

Transmission

- **Directly**→most commonly through cuts, abraded skin, but also can occur through mucous membranes, especially the conjunctival and oral mucosa
- **Indirectly**→(Less commonly than the direct route) through contaminated water and food.

The disease is biphasic:



- ***Clinical findings :***
- Leptospirosis is characterized by a broad spectrum of clinical manifestations, varying from asymptomatic, self-limited mild flu-like infection to fulminant, fatal disease (**Weil's Syndrome)** which is present in just about 1% of cases.

  • In the leptospiremic phase: **systemic symptoms** appear on the patient like fever, chills, rigors, nausea, vomiting, abdominal pain, musculoskeletal symptoms and night sweats. When the second phase starts, the hallmark would be the pathology of the affected organ as previously mentioned (e.g. liver failure).

  • Remarkable redness of the eye occurs if the infection was through the conjunctiva.

- ***Diagnostic laboratory tests :***

  • A. **Specimens**

  Specimens consist of blood, CSF, or urine and tissues for microscopic examination and culture.

  • B. **Microscopic Examination**

  Dark-field examination or thick smears stained by the Giemsa technique.

• C. **Culture**

Whole fresh blood ,CSF or urine or crushed tissue can be cultured. Leptospires grow best under aerobic conditions at 28–30 C in semisolid medium (eg, **Ellinghausen-McCullough- Johnson- Harris EMJH**) in 10 mL test tubes with 0.1% agar and 5-fluorouracil.

Growth is slow. ,and cultures should be kept for at least 8 weeks .

• D. **Serology**

The diagnosis of leptospirosis in most cases is confirmed serologically with microscopic agglutination test (**MAT)** and ELISA.

- ***Treatment and immunity:***
- Treatment of mild leptospirosis should be with **oral doxycycline,** ampicillin, or amoxicillin.

  • Severe leptospirosis should be treated with **IV penicillin** as soon as the diagnosis is consider.

  • Serovar-specific immunity follows infection, but reinfection with different serovars may occur.

- ***Prevention and control :***

- Leptospirae is excreted in **urine** both during the active illness and during the asymptomatic carrier state.

  • Leptospirae remain viable in stagnant water for several weeks; drinking, swimming, bathing, or food contamination may lead to human infection. Thus, people most likely to come in contact with water contaminated by rats (e.g. miners, sewer workers, farmers, and fishermen) run the greatest risk of infection ⇒ they should avoid exposure to urine and tissues from infected animals through proper eyewear, footwear, and other protective equipment. Targeted rodent control strategies could also be considered.

  • Vaccines for agricultural and companion animals are generally available, and their use should be encouraged. **No vaccines for humans.**

Mycobacterium Tuberculosis (M. tb)

It was not until the 19th century, when Robert Koch utilized s new staining method (ZN stain) and applied it to sputum from patients discovering the causal agent of the disease Tuberculosis (TB); Mtb or Koch bacillus

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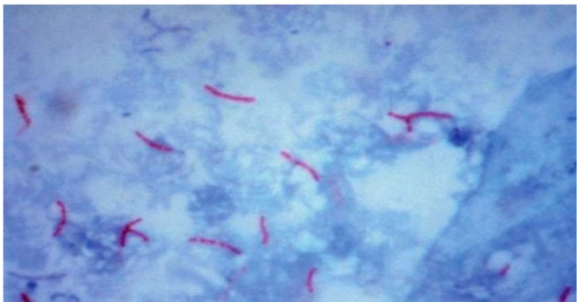
Tuberculosis , **consumption**(consume patients ,weight loss), **white plaque** (extreme pallor • seen among patients)

The family mycobacterium tuberculosis complex(MTC) can cause Tuberculosis (TB) in • humans and other livings

It includes M. tuberculosis (Mtb), Mycobacterium africanum, •. Mycobacterium bovis, Mycobacterium microti, Mycobacterium caprae, Mycobacterium pinnipedii, Mycobacterium suricatte, Mycobacterium mungi, Mycobacterium dassie, Mycobacterium oryx and Mycobacterium canetti



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. Mtb is a slow growing ,obligate aerobe, facultative intra- cellular bacterium •
.Non-spore forming, non-motile acid fast bacilli •

⇒ TB is considered an airborne infectious disease although M. tuberculosis complex organisms can be spread through un-pasteurized milk, direct inoculation and other means.

May be weakly gram **positive but gram stain is not applicable so acid fast stain (ZN stain)** is used due to the complexity of the cell wall which is composed mainly of lipids: wax D, mycolic acid, cord factor (trehalose dimycolate)→main virulence factors.

⇒ NOTE: The marked pathology of tuberculosis is **granuloma formation**

*TB remains a leading cause of infectious diseases morbidity and mortality. In 2015, an estimated 10.4 million new TB cases were seen worldwide. However, in Jordan, the number of TB cases has dropped in the last 2 decades to 25 cases per 100,000 individuals now.

- There are two new strains of the tb : 1- **MDR** = multi drug resistance
  2- **XDR** = extensively drug resistance

- At least the treatment course should be continue to **6** months

Tuberculosis :

- **Pulmonary tuberculosis** makes up 80-90% of TB cases: The primary site of TB is usually the lungs, from which it can get disseminated into other parts of the body.

⇒ Other routes of spread include contiguous involvement from adjacent tuberculous lymphadenopathy or primary involvement of extra-pulmonary organ.

Extra-pulmonary tuberculosis makes up 10-20% of TB cases: bacteria can attack any ☆ part of the body such as the pleura, lymph nodes (Scrofula), pericardium, kidney, spine **(Pott disease),** brain, meninges (tuberculous meningitis especially in children) and abdomen (abdominal tuberculosis)

:Abdominal tuberculosis comprises 5% of all cases of TB ➔

Abdominal TB, can be a source of significant morbidity and mortality and is usually ▪ diagnosed late due to its nonspecific clinical presentation

.

Abdominal TB usually occurs in four forms: **tuberculous lymphadenopathy, ▪ peritoneal tuberculosis, gastrointestinal (GI) tuberculosis and/or visceral tuberculosis involving the solid organs**

- ***Pathogenesis :***
- Can occur by the following ways:

《1》 Ingestion of tuberculous mycobacteria (as with ingestion of sputum or undercooked meat or unpasteurized milk contaminated with M. bovis which lives in cattle)

《2》 Hematogenous or lymphatic spread to the GIT from another foci. E.g. In the setting of active pulmonary TB or **miliary TB**

《3》 Direct spread from a contiguous (adjacent) location. E.g. from the peritoneum (when there is peritoneal tuberculosis) or retrogradely from fallopian tubes or from the ovaries

《4》 May occur via reactivation of latent TB infection

❖ More details were written in the slides without mentioned it in the lecture :

The mucosal layer of the GI tract can be infected with the bacilli, leading to the formation of epithelioid tubercles in the lymphoid tissue of the submucosa.
After 2-4 weeks, caseous necrosis will occur in the tubercles, causing ulceration of the overlaying mucosa which will spread into the deeper layers and adjacent lymph nodes as well as the peritoneum.
Rarely, the bacilli can enter into the portal circulation or the hepatic artery thus involving organs such as the liver, pancreas or spleen

Clinical findings :

The clinical presentation tends to be non-specific, with abdominal pains and general complaints

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- Although any portion of the gastrointestinal tract may be affected, the terminal ileum and the cecum are the sites most commonly involved. Abdominal pain (at times similar to that associated with appendicitis) and swelling, obstruction, hematochezia, and a palpable mass in the

abdomen are common findings at presentation. Fever, weight loss, anorexia, and night sweats are also common

Laboratory diagnostic methods:

A-smear microscopy :

Three specimens from each patient with suspected TB should be examined .microscopically for **Acid Fast Bacilli AFB (using Ziehl-Neelsen stain)**

- Mycobacteria can also be examined by yellow fluorescence after staining with auramin

B. Culture

- Both liquid and solid mycobacterial cultures should be performed for every specimen, examples: Lowenstein-Jensen or Middlebrook **7H10**, Radiometric broth .culture (**BACTEC** radiometric system), mycobacterial growth indicator tube (**MGIT**)

Agar should be incubated for at **least 4-5 weeks** before we start seeing colonies on the agar plate and up to 8 weeks before announcing that the culture is negative for M. Tb

- Culture for acid fast bacilli is the most specific test for TB and allows direct identification and determination of susceptibility of the causative organism

A nucleic acid amplification test (NAAT), Tuberculin skin tests (TSTs), Interferon-gamma release assays (IGRAs) are commonly used as well.but they have low. sensitivity and don't give us accurate results.

Treatment:

- The course of TB treatment depends on whether the individual is in the latent or active stage, and on his or her probability of risk.
- Treatment of TB usually involves a drug cocktail, or a mixture of multiple drugs, with an intensive initial 2-month phase followed by a slower 4- to 6-month continuation phase the main anti-tuberculosis drugs used in the chemotherapy of TB are: isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and either ethambutol (EMB) or streptomycin (SM).
- Isoniazid preventive therapy IPT is the recommended treatment for LTBI but the regimen's main drawback is the duration of therapy

latent

Prevention:

- The best way to prevent TB is to diagnose and isolate infectious cases rapidly and to administer appropriate treatment until patients are rendered noninfectious (usually 2–4 weeks after the start of proper treatment) and the disease is cured.

- Additional strategies include BCG vaccination and treatment of persons with LTBI who are at high risk of developing active disease.

- Mycobacterium bovis Bacillus Calmette–Guérin (BCG), an attenuated vaccine derived from M. bovis, is the only licensed vaccine against tuberculosis (TB)

صورة تعود لمسجد قبة الصخرة عام ١٩٠٠ ميلادي في عاصمة فلسطين القدس ويظهر فيها ابناءها وحراسها

Good luck