MINISTRY OF HEALTH SERVISE OF UKRAINE
ZAPOROZHYE STATE MEDICAL UNIVERSITY

THE CHAIR OF MICROBIOLOGY, VIROLOGY AND IMMUNOLOGY

Mycobacterium tuberculosis
Mycobacterium leprae

The methodical manual

on microbiology, virology and immunology

for medical the students of 2 - 3 courses

of the international faculty

Zaporizhzhia

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M 99 **Mycobacterium tuberculosis** Mycobacterium leprae : the methodical manual on microbiology, virology and immunology for the students of 2-3 courses of the medical faculty / Yeryomina A. K. [et al.]. – Zaporizhzhia, 2016. – 80 p.

The methodical manual for practical lessons on microbiology, virology, immunology for the medical students of II-III year of the study are approved by the Central Methods Board of ZSMU as a methodical manual on practical lessons for students of the medical faculty.

The independent practical work of students is an important part of the syllabus in the course of microbiology, virology and immunology. It helps students to study this fundamental subject.

The systematic independent work enables to reach the final goal in the students' education. It is also important while preparing the students for their future clinic work with patients.

These theoretical material, questions and tests help students to get ready for examination.

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
ЗАПОРІЗЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ

Кафедра мікробіології, вірусології та імунології

Мікобактерії туберкульозу та лепри

Навчальний посібник

для студентів II-III курсів міжнародного факультету,

спеціальність «Лікувальна справа»

Запоріжжя

2016

Навчальний посібник затверджено на засіданні Центральної методичної Ради ЗДМУ (протокол № від 20 р.) та рекомендовано для використання в навчальному процесі.

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Мікобактерії туберкульозу та лепри : навч. посібник для іноземних студентів II-III курсів медичного ф-ту, спеціальність «Лікувальна справа» / Єрьоміна А.К. [та ін.]. – Запоріжжя, 2016. – 81 с.

Навчальний посібник з мікробіології, вірусології та імунології для іноземних студентів II-III курсів медичного факультету спеціальності «Лікувальна справа».

Mycobacteria of Tuberculosis and Leprosy

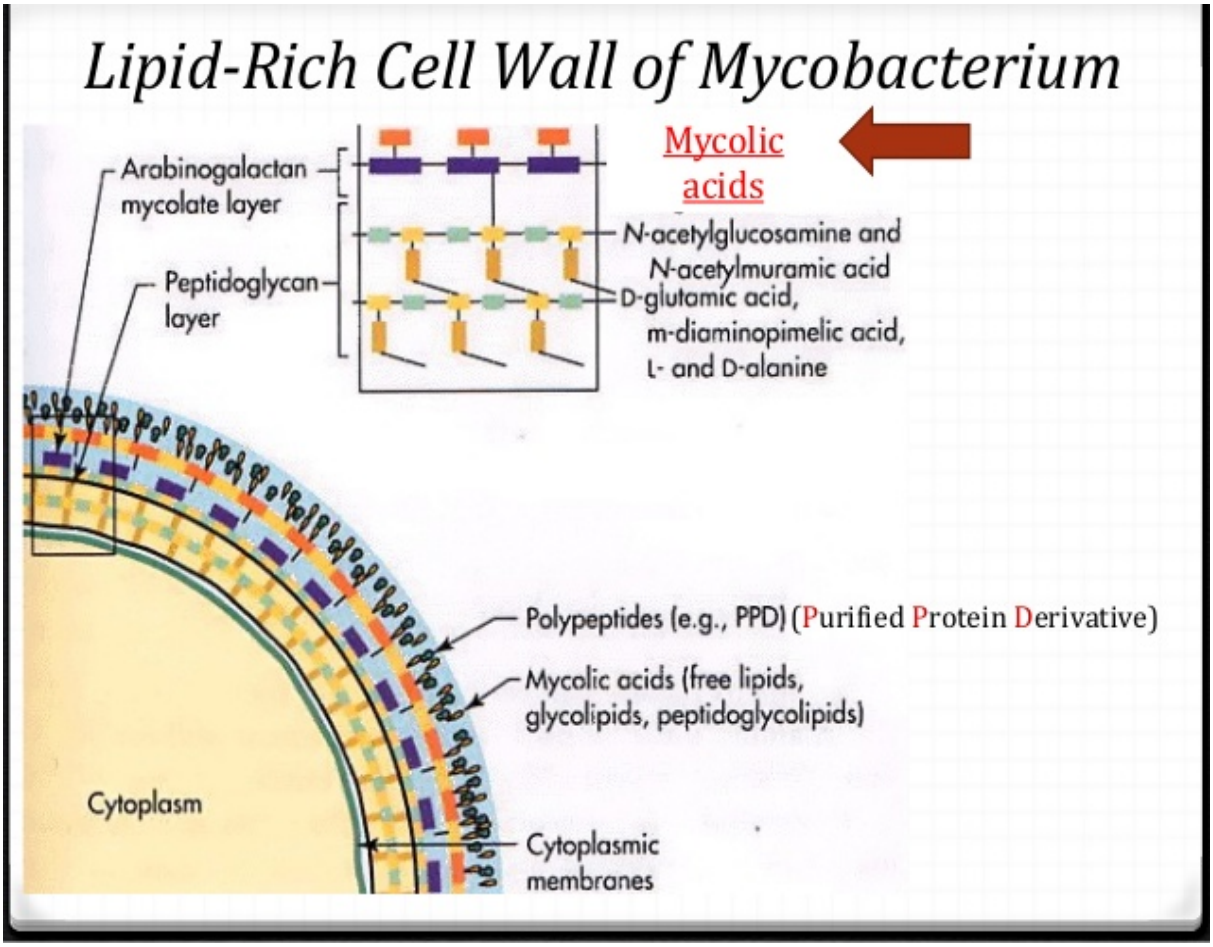
General characteristics of *Mycobacteria*

The genus *Mycobacterium* is the only genus of the family *Mycobacteriaceae.* The genus includes obligate parasites, opportunistic pathogens and saprophytes.

Mycobacteria are slender rods that sometimes show branching filamentous forms resembling fungal mycelium. Hence the name "mycobacteria", meaning fungus-like bacteria. They do not stain readily but once stained, resist decolorization with dilute mineral acids.

The mycobacteria are rod-shaped, aerobic bacteria that do not form spores. They are 'acid-fast' bacilli, once stained the mycobacteria resist decolorization by acid or alcohol.

Mycobacteria have cell walls with a high lipid content that includes waxes having characteristic mycolic acids with long, branched chains.



Several mycobacteria, distinct from human or bovine tubercle bacilli, which have been isolated on occasion from human pathological material, have been grouped together under the loose term "atypical mycobacteria".

Unlike tubercle bacilli which are strict parasites, atypical mycobacteria may occur in soil, water and other sources.

Many species are slow-growing organism. There are more than 50 mycobacterium species.

Many mycobacterium species are **saprophytes** that very rarely cause disease in humans (e.g., *Mycobacterium gordonae, M. flavescens, M. fallax, M. gastri).* These mycobacterium species usually contaminate environment.

Other mycobacteria species can infect humans.

Some of them are **primary** (frank) **pathogens** causing disease in a healthy host:

(1) *Mycobacterium tuberculosis* is a very important pathogen of humans, it causes **tuberculosis;**

(2) *Mycobacterium leprae* causes **leprosy. Atypical mycobacteria,** such as *Mycobacterium avium-intracellulare (M. avium* complex, MAC), are **opportunistic pathogens.**

They infect immunocompromised persons (e.g., patients with AIDS). They may occasionally cause disease in patients with normal immune system.

Humans and guinea pigs are highly susceptible to *M tuberculosis* infection; fowl and cattle are resistant.

M. tuberculosis and *M. bovis* are equally pathogenic for humans.

The route of infection (respiratory or intestinal) determines the pattern of lesions.

Some 'atypical' mycobacteria (e.g., *M kansasii)* produce human disease indistinguishable from tuberculosis.

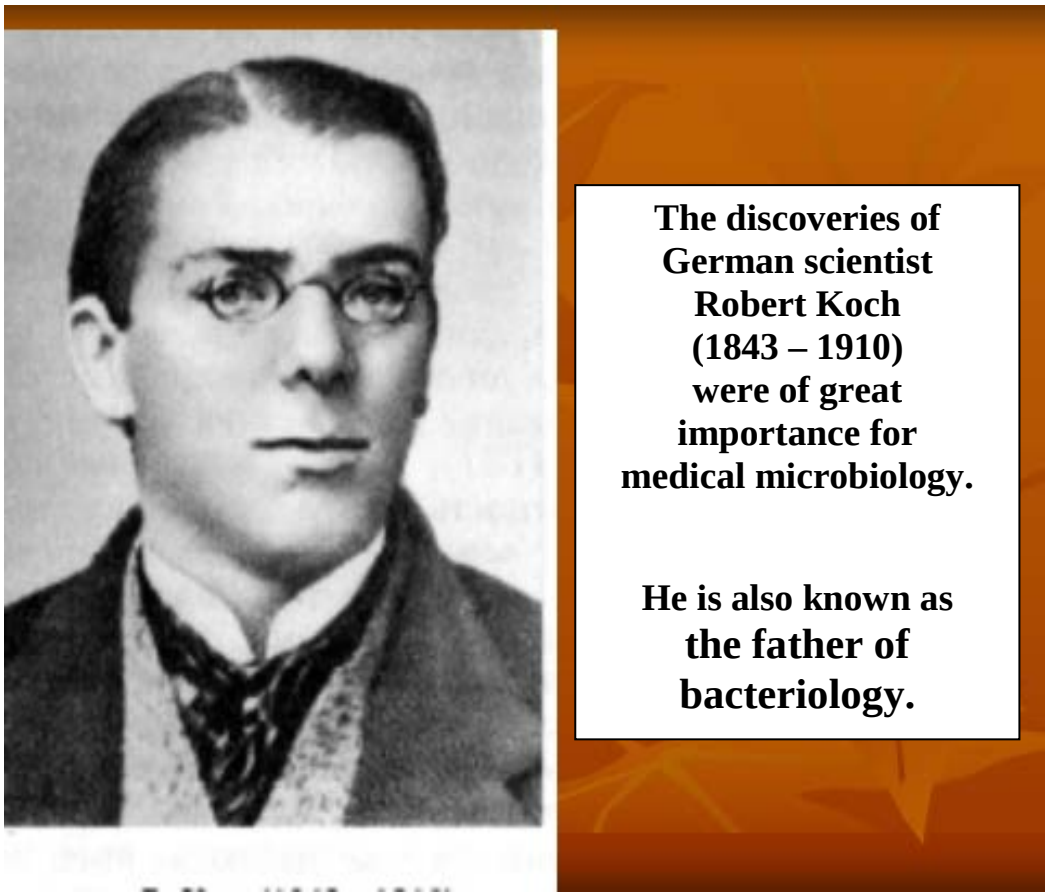
Others (e.g., *M fortuitum)* are opportunists or cause only surface lesion.

History

The 1st member of this genus to be identified was the leper bacillus discovered by Hansen in 1868.

M. tuberculosis, then known as the "tubercle bacillus", was first described on 24 March 1882 by **Robert Koch,** who subsequently received the Nobel Prize in physiology or medicine for this discovery in 1905; the bacterium is also known as "Koch's bacillus".

R. Koch isolated the mammalian tubercle bacillus and proved its causative role in tuberculosis by satisfying Koch's postulates.



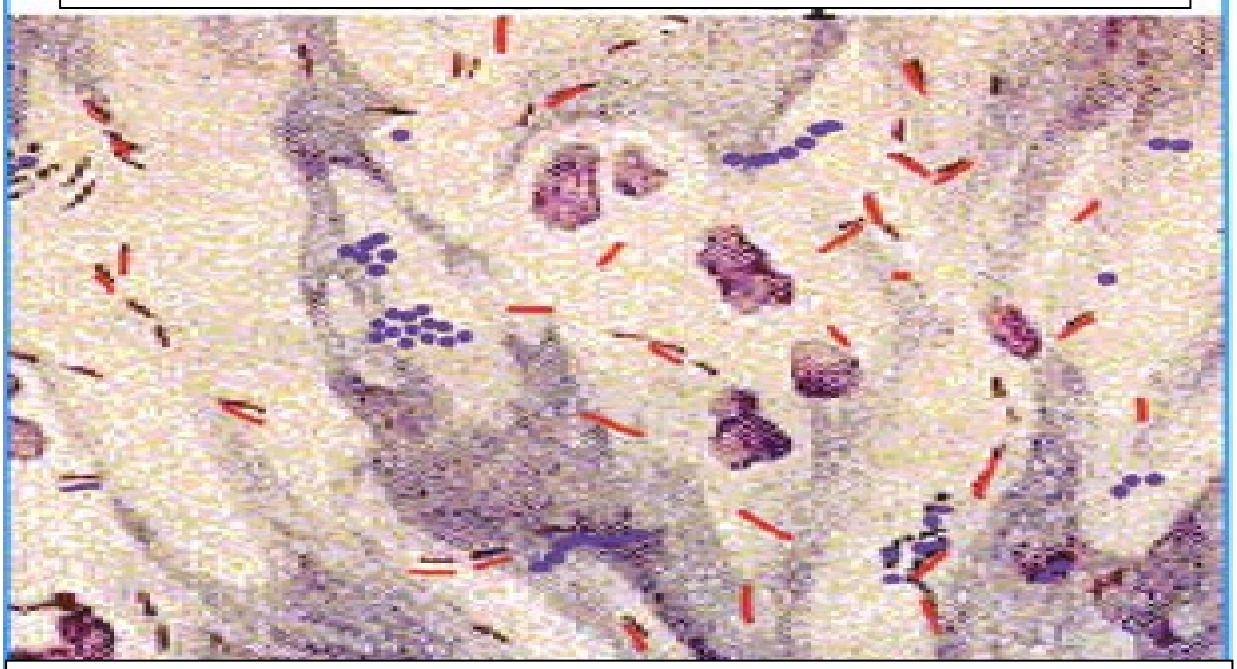
In 1720, though, the history of tuberculosis started to take shape into what is known of it today; as the physician Benjamin Marten described in his *A Theory of Consumption*, tuberculosis may be caused by small living creatures transmitted through the air to other patients

Mycobacterium tuberculosis is an obligate pathogenic bacterial species and the causative agent of most cases of tuberculosis.

M. tuberculosis has an unusual, waxy coating on its cell surface (primarily due to the presence of mycolic acid), which makes the cells impervious to Gram staining; M. tuberculosis can appear gram-negative and gram-positive in clinical settings.

The Ziehl-Neelsen stain, or acid-fast stain, is used instead.

M. tuberculosis ***(stained red)*** *in tissue* ***(blue)***



Mycobacterium tuberculosis

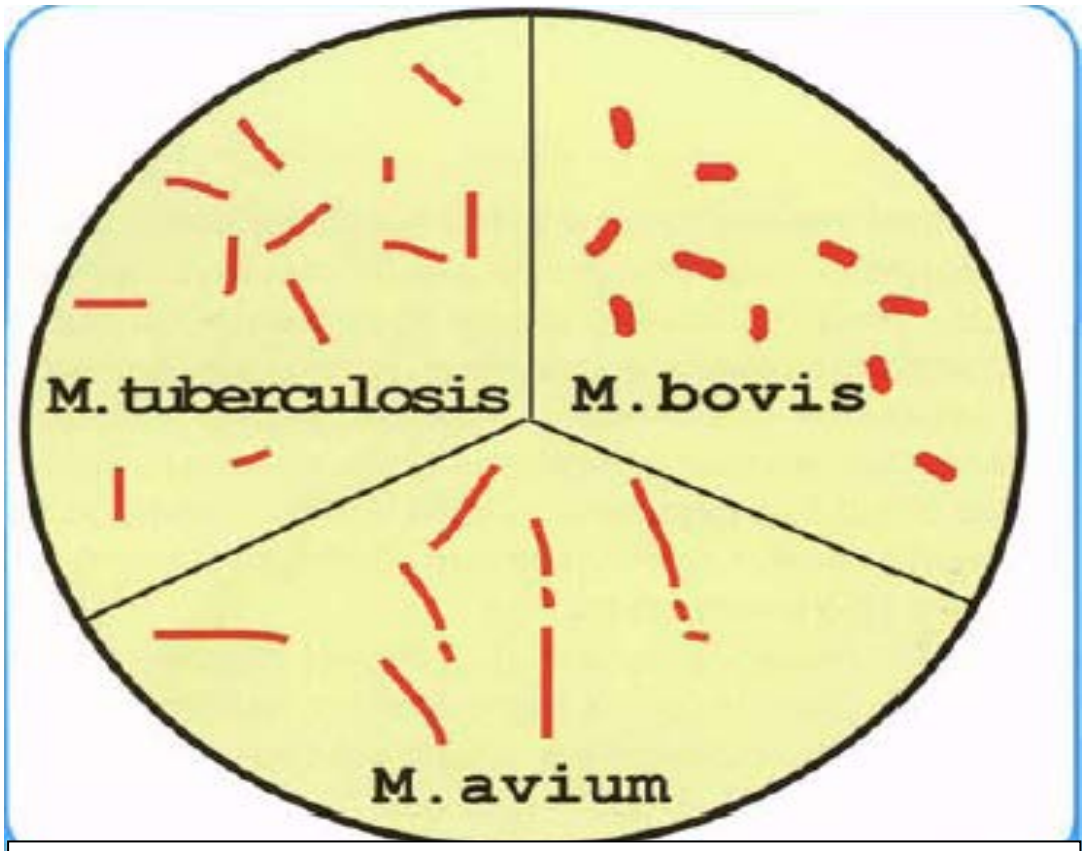
Staining by Ziehl-Neelsen

Tuberculosis in humans was subsequently shown to be caused by 3 types of the bacillus - the human, bovine and bird types, designated

Mycobacterium tuberculosis,

M.bovis,

M.avium respectively.



Types of mycobacterium

Antigenic structure.

Many antigens have been identified in mycobacteria. Group specificity is due to polysaccharide and type specificity to protein antigens. Following infection by tubercle bacilli, delayed hypersensitivity is developed to the protein of the bacillus (tuberculin).

Tuberculosis patients possess circulating antibodies against the polysaccharide, protein and phosphatide antigens of the tubercle bacillus demonstrable by the use of the sensitized erythrocyte, tanned and protein coated erythrocyte, and phosphatide kaolin agglutination tests.

These antibodies are of no diagnostic value and appear to be irrelevant in immunity.

Toxin production. The tubercle bacillus does not appear to contain or produce a toxin. In 1890 Koch prepared a protein extract of the tubercle bacillus.

This was called "original" or "old tuberculin". Koch 1st employed "old tuberculin" in the treatment of tuberculosis but it was soon given up as it was not only not beneficial but also caused serious illness in some patients due to the 'focal' and 'systemic' components of the Koch phenomenon.

Several methods had been used for tuberculin testing but the graded intradermal technique of *Mantoux* (1910) is employed in standard practice.

In the Mantoux test, using a tuberculin syringe, raising a wheal. The site is examined 48-72 hours later and the diameter of induration measured transversely to the long axis of the forearm.

Erythema is not taken into account. Induration of diameter 10 mm or more is considered positive; 5 mm or less considered negative.

A positive tuberculin test indicates hypersensitivity to tuberculoprotein, denoting infection with the tubercle bacillus or BCG immunization, recent or past, with or without clinical disease.

Tuberculin testing may be used as an aid in diagnosing active infection in infants and young children, to measure the prevalence of infection in a community, to select susceptibles for BCG vaccination or as an indication of successful vaccination.

Cultivation

The bacilli grow slowly, the generation time in vitro being 14-15 hours. Colonies appear only in about 2 weeks and sometimes may be delayed up to 6-8 weeks.

Optimum temperature is 37°C and growth does not occur below 25°C or above 40°C. Optimum pH is 6.4-7.0.

M *tuberculosis* is an obligate aerobe, while *M. bovis* is microaerophilic on primary isolation, becoming aerobic on subculture.

Growth is stimulated by 5-10 % CO_2. *M .tuberculosis* grows luxuriantly in culture as compared to M. *bovis* which grows sparsely.

Tubercle bacilli do not have exacting growth requirements but are highly susceptible to even traces of toxic substances like fatty acids in culture media. The toxicity is neutralized by serum, albumin or charcoal.

R.Koch originally grew the bacillus on heat-coagulated bovine serum. Several media, both solid and liquid, have been described for the cultivation of tubercle bacilli.

The solid media contain egg (Lowenstein-Jensen, Petragnini), serum (Loeffler′s serum slope) or potato (Pawlowsky's).

The solid medium most widely employed for routine culture is the **Lowenstein-Jensen medium** without starch, as recommended by the International Union Against Tuberculosis (IUAT).

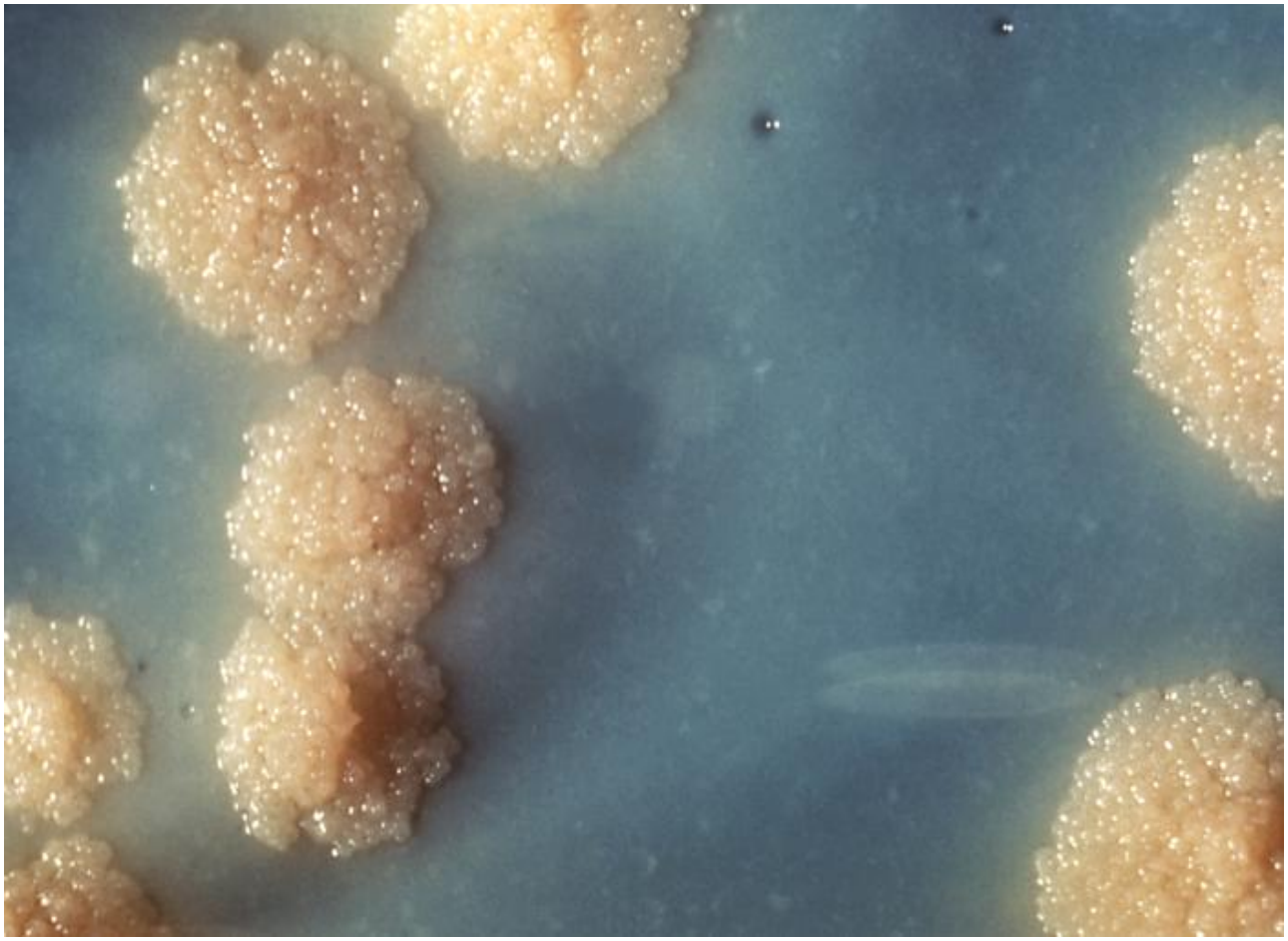
This consists of coagulated hen's egg, mineral salt solution, asparagine and malachite green, the last acting as a selective agent inhibiting other bacteria.

A simple medium containing only eggs, malachite green and coconut water has been reported to be a useful and cheap alter - native to the Lowenstein-Jensen medium.

On solid media *M. tuberculosis* forms dry, rough, raised, irregular colonies with a wrinkled surface.

They are creamy white initially, becoming yellowish or buff colored later. They are tenacious and not easily emulsified.

The colonies of *M. bovis* are in comparison, flat, smooth, moist and white, breaking up easily when touched.



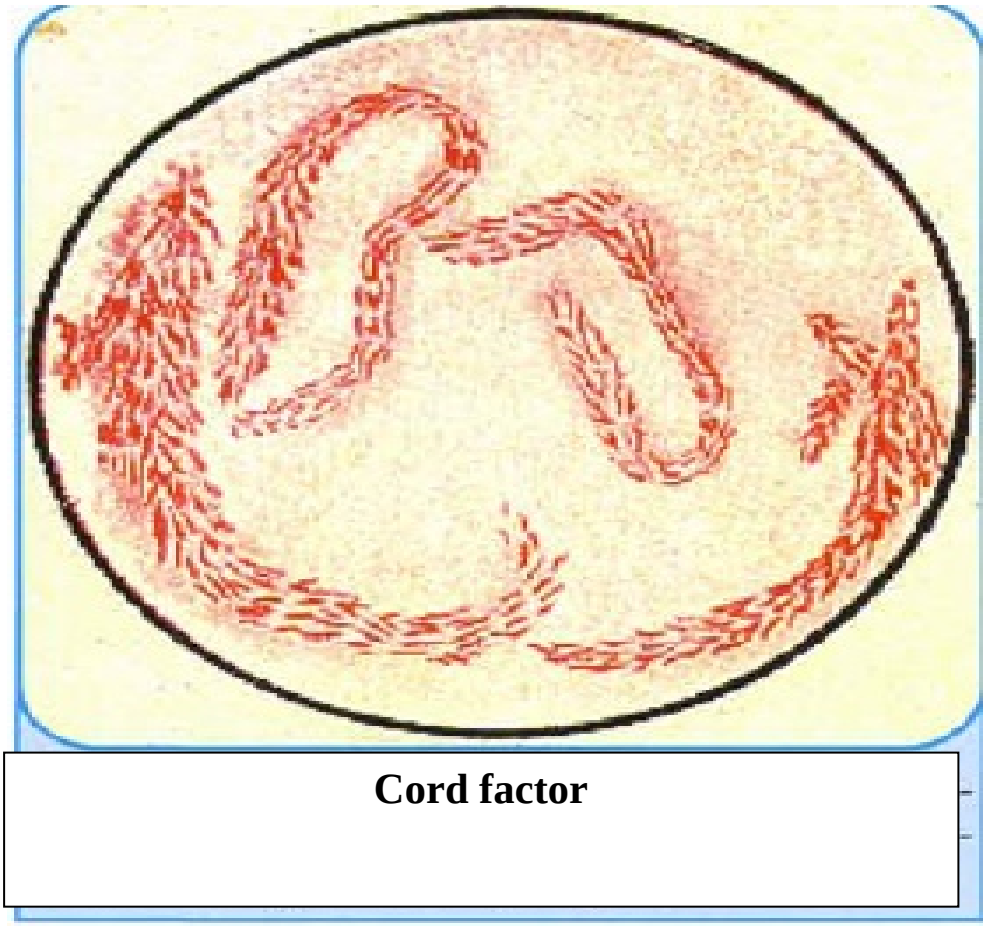
M. tuberculosis bacterial colonies

on the Lowenstein – Jensen medium

Tubercle bacilli may also be grown in chick embryos and in tissue culture.

Virulent strains tend to form long serpentine cords in liquid media, while avirulent strains grow in a more dispersed fashion.

The cord factor by itself is not responsible for virulence. It is present in some non-pathogenic species of mycobacteria as well.



Cord factor

Laboratory diagnosis of tuberculosis

Tuberculin test: This is a test to find past or present tuberculosis infection based on a positive skin reaction.

Tuberculin may be placed into the skin by injection, scratch, or puncture. If a raised, red, or hard zone forms around the tuberculin test site in 48-72 hours, the person is said to be sensitive to tuberculin, and the test is read as positive.

A positive tuberculin test indicates that an individual has been infected in the past and continues to carry viable tubercle bacilli in some tissue.

Mycobacteria may be either pathogenic or avirulent (e.g., BCG vaccine strain). A negative tuberculin reaction does not rule out a diagnosis of earlier or active tuberculosis.

Two types of tuberculin are known:

(1) old tuberculin, a concentrated filtrate of broth in which tubercle bacilli have grown for 6 weeks, contains tuberculoproteins and various other constituents of mycobacteria and of growth media;

(2) a purified protein derivative (PPD) obtained by chemical fractionation of components of tubercle bacilli (new tuberculin).

'Tuberculin units' (TU) are used to standardize biological activity of tuberculin. The TU is defined as the activity contained in a specified weight of Seibert's PPD Lot No. 49608 in a specific buffer (PPD-S, the standard for tuberculin against which the potency of all products must be established).

Usually material containing 5 TU (intermediate-strength tuberculin) is injected. In persons suspected of extreme hypersensitivity, material containing 1 TU (first-strength tuberculin) is used.

Material containing 250 TU (second-strength tuberculin) is administered only if the reaction to 5 TU is negative. The volume of tuberculin is ordinarily 0.1 mL injected intracutaneously. The skin test should be read in 48 or 72 hours.

Variants of reactions to tuberculin:

(1) The skin test is considered positive if the injection of 5 TU is followed by induration of 10 mm or more in diameter. Induration, edema, and erythema are usually developed in 24-48 hours in an individual who has had a primary infection with mycobacteria (central necrosis occurs with very intense reaction). The tuberculin test becomes positive within 4-6 weeks after infection or injection of BCG vaccine (avirulent organisms). After BCG vaccination, a positive test may last for 3-7 years. Only the elimination of viable tubercle bacilli results in reversion of

the skin test to negative. Positive test means that the person is hypersensitive to tuberculin.

(2) The skin test is considered negative if there is no or very limited reaction to tuberculin. It may be negative if an individual has not had contact with mycobacteria; or in the presence of tuberculosis infection when 'anergy' develops due to overwhelming tuberculosis, measles, Hodgkin's disease, sarcoidosis, AIDS, or immunosuppression.

Diagnostic laboratory tests:

A positive tuberculin test does not prove the presence of active tuberculosis. Isolation of tubercle bacilli only proves the presence of the active disease due to mycobacteria.

Specimens: fresh sputum, pleural fluid, gastric washings, urine, cerebrospinal fluid, joint fluid, blood, biopsy material, or other materials.

Specimens processing is recommended. This consists of decontamination and concentration.

(1) Specimens from non-sterile sites (e.g., sputum) should be liquefied (with JV-acetyl-L-cysteine), decontaminated with NaOH (kills other bacteria and fungi), neutralized with buffer, and concentrated by centrifugation;

(2) Specimens from normally sterile sites (e.g., cerebrospinal fluid) do not need the decontamination procedure. They can be centrifuged, microscopically examined, and cultured.

Microscopic examination:

Specimens (processed by decontamination and concentration, or unprocessed) should be used for staining.

(1) Ziehl-Neelsen staining is used to examine for acid-fast bacilli such materials as sputum, exudates, or others (but not gastric washings, and urine where

saprophytic acid-fast mycobacteria may be present). If acid-fast organisms are found in a specimen, this is presumptive evidence of mycobacterial infection. Smears stained by Ziehl-Neelsen technique can be examined under the light microscope (for negative result at least 100 fields have to be examined).

The sensitivity of the test is low (at least 10,000 acid fast bacilli per 1 ml of sputum).

Several modifications of concentration methods are used to decontaminate sputum and concentrate acid-fast bacilli:

homogenization methoc (sputum is treated with 1% sodium hydroxide, is mixed for 10-20 min, centrifuged and the sediment should be neutralized and used for acid fast staining;

flotation method (after homogenization the sputum is heated, xylol and mixed for 10 min.

Xylol drops absorb Mycobacteria and form flotation layer on the top of the liquid and the top layer can be transferred to a single slide).

(2) Fluorescence microscopy with auramine or rhodamine stain is more sensitive than acid-fast stain. A confirmatory acid-fast stain is necessary if the fluorescent microscopy is positive.

Culture: Processed specimens should be cultured directly onto selective and nonselective media.

Nonselective media of routine use are semisynthetic agar media (e.g., Middlebrook 7H10 and 7H11), inspissated egg media (e.g., Lowenstein-Jensen), and broth media (e.g., Middlebrook 7H9 and 7H12).

Nonselective media with added antibiotics are used as selective media. The selective broth culture often is the most sensitive and rapid method.

A selective agar media (e.g., Middlebrook 7H10 and 7H11) or Lowenstein-Jensen media with added antibiotics should be inoculated in parallel with broth media cultures and incubated at 37° C in 5-10% CCH for up to 8 weeks.

Incubation may be prolonged when presence of slowly growing atypical mycobacteria is suspected (cultures are negative in the setting of a positive acid-fast stain).

In this case, a new set of inoculated media should be incubated at a lower temperature (22-33° C).

Both sets (the set incubated at 37° C and the new one) should be incubated for 12 weeks.

Blood for culture of mycobacteria (usually M. avium complex) should be anticoagulated and processed by one of three methods:

(1) commercially available lysis centrifugation system;

(2) inoculation into commercially available broth media specifically designed for blood cultures;

(3) centrifugation of the blood and inoculation of the white blood cell buffy coat layer, with or without deoxycholate lysis of the cells, into broth culture. Solid media can be used in parallel.

Identification of isolated mycobacteria as to species (first of all, separation of M tuberculosis from all other mycobacteria) includes conventional and other methods.

A. Conventional methods require 6-8 weeks for identification of mycobacteria. These methods include observation of:

(1) Rate of growth - separates the which grow in < 7 days (e.g., M.fortuitum, M. chelonae, M. abscessus, M. smegmatis, M. vaccae, and M. phlei), from other mycobacteria;

(2) Colony morphology;

(3) Pigmentation subdivides all mycobacteria to:

(i) photochromogens, which produce pigment in light but not in darkness (e.g., M. asiaticum, M. kansasii, M. marinum, and M. simiae);

(ii) scotochromogens, which produce pigment when growing in the dark (e.g., M. flavescens, M. gordonae, M. scrofulaceum, and M. szulgai); and

(iii) nonchromogem, which are nonpigmented or have light tan or buff-colored colonies (e.g., M. avium complex, M. haemophilum, M. celatum, M. gastri, M. nonchromogenicum, M. ulcerans);

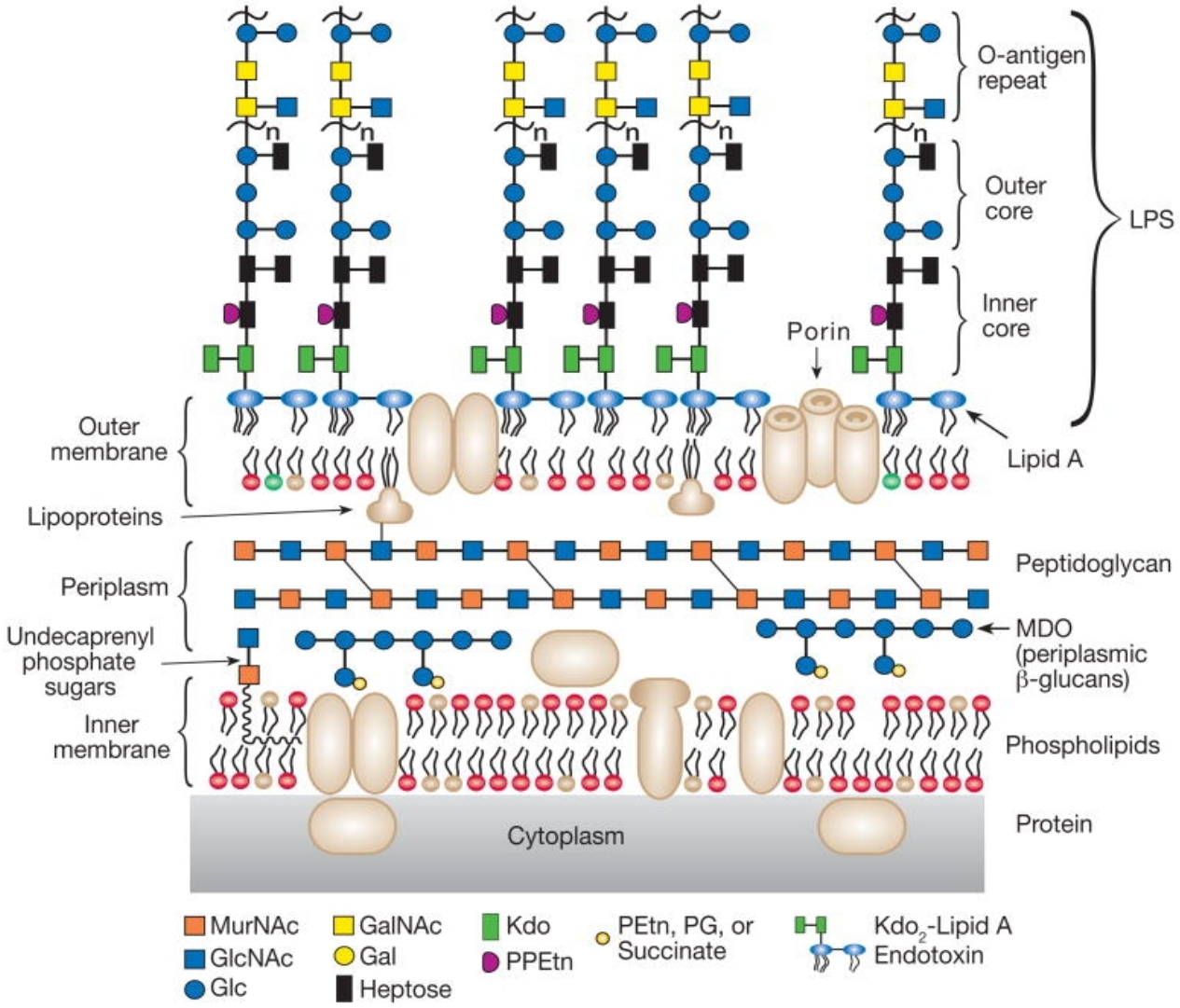
(4) Biochemical characteristics: production of niacin (positive as to M. tuberculosis), nitrate reduction (positive as to M. tuberculosis), catalase test (negative or weakly positive as to M tuberculosis), peroxidase test (positive as to M. tuberculosis), aryl sulphatase test (negative or weakly positive as to M. tuberculosis), and others.

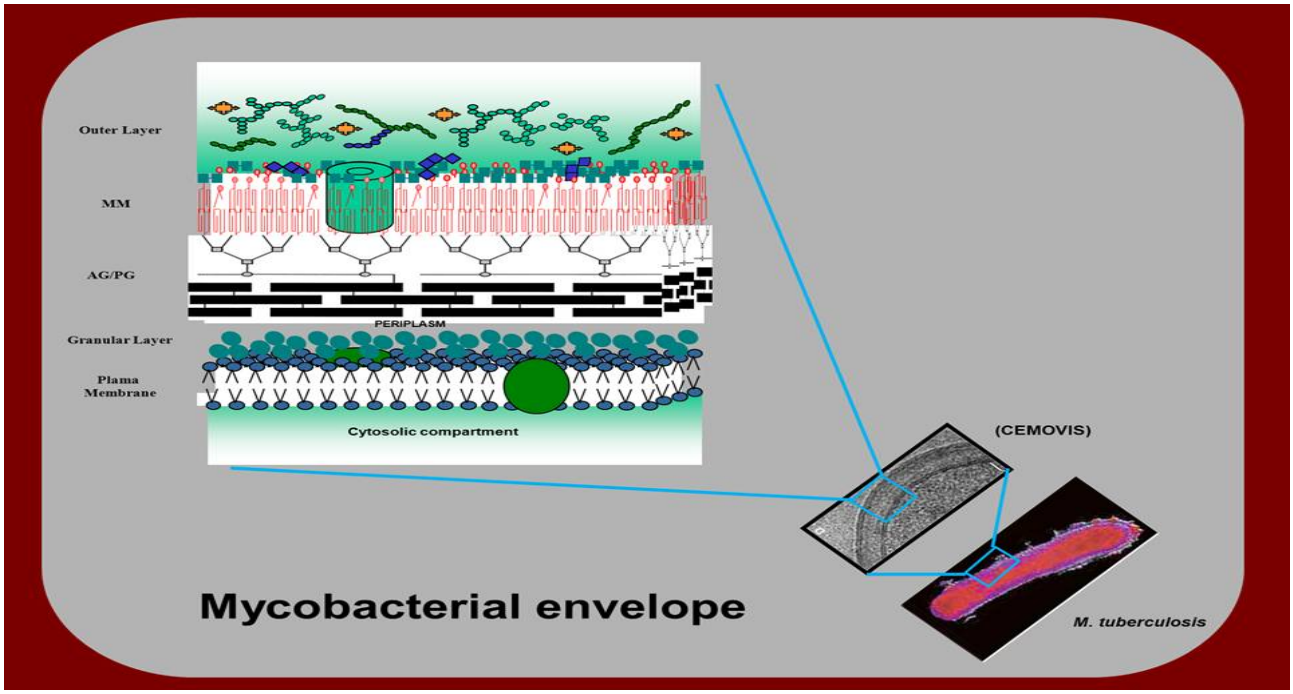
Pathophysiology

M. tuberculosis requires oxygen to grow and can be cultured in the laboratory.

It does not retain any bacteriological stain due to high lipid content in its wall, hence Ziehl-Neelsen staining, or acid-fast staining, is used.

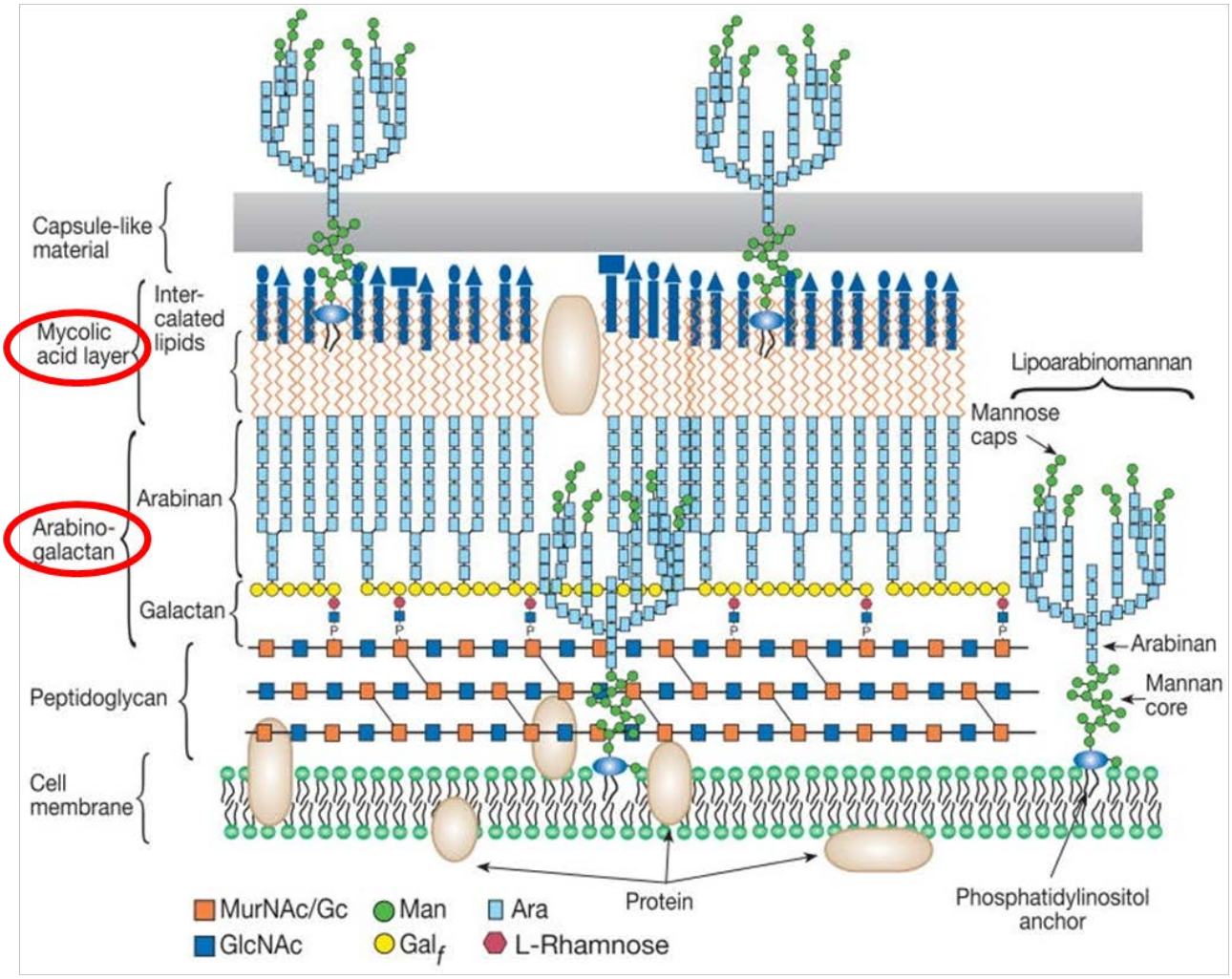
Mycobacteria have an outer membrane.





M. tuberculosis divides every 15–20 hours, which is extremely slow compared to other bacteria, which tend to have division times measured in minutes (*Escherichia coli* can divide roughly every 20 minutes).

It is a small bacillus that can withstand weak disinfectants and can survive in a dry state for weeks. Its unusual cell wall, rich in lipids (e.g., mycolic acid), is likely responsible for this resistance and is a key virulence factor.



Humans are the only known reservoirs of *M. tuberculosis*. There are misconceptions that *M. tuberculosis* can be spread by shaking hands, making contact with toilet seats, sharing food or drink, sharing toothbrushes, or kissing.

M. tuberculosis can only be spread through air droplets originating from a person either coughing, sneezing, speaking, or singing that has the disease.

When in the lungs, M. tuberculosis is taken up by alveolar macrophages, but they are unable to digest and eradicate the bacterium.

Its cell wall prevents the fusion of the phagosome with the lysosome, which contains a host of antimycobacterial factors.

Specifically, M. tuberculosis blocks the bridging molecule, early endosomal autoantigen 1 (EEA1); however, this blockade does not prevent fusion of vesicles filled with nutrients. Consequently, the bacteria multiply unchecked within the macrophage.

The bacteria also carry the UreC gene, which prevents acidification of the phagosome. In addition, production of the diterpene isotuberculosinol prevents maturation of the phagosome.

The bacteria also evade macrophage-killing by neutralizing reactive nitrogen intermediates.

Protective granulomas are formed due to the production of cytokines and upregulation of proteins involved in recruitment.

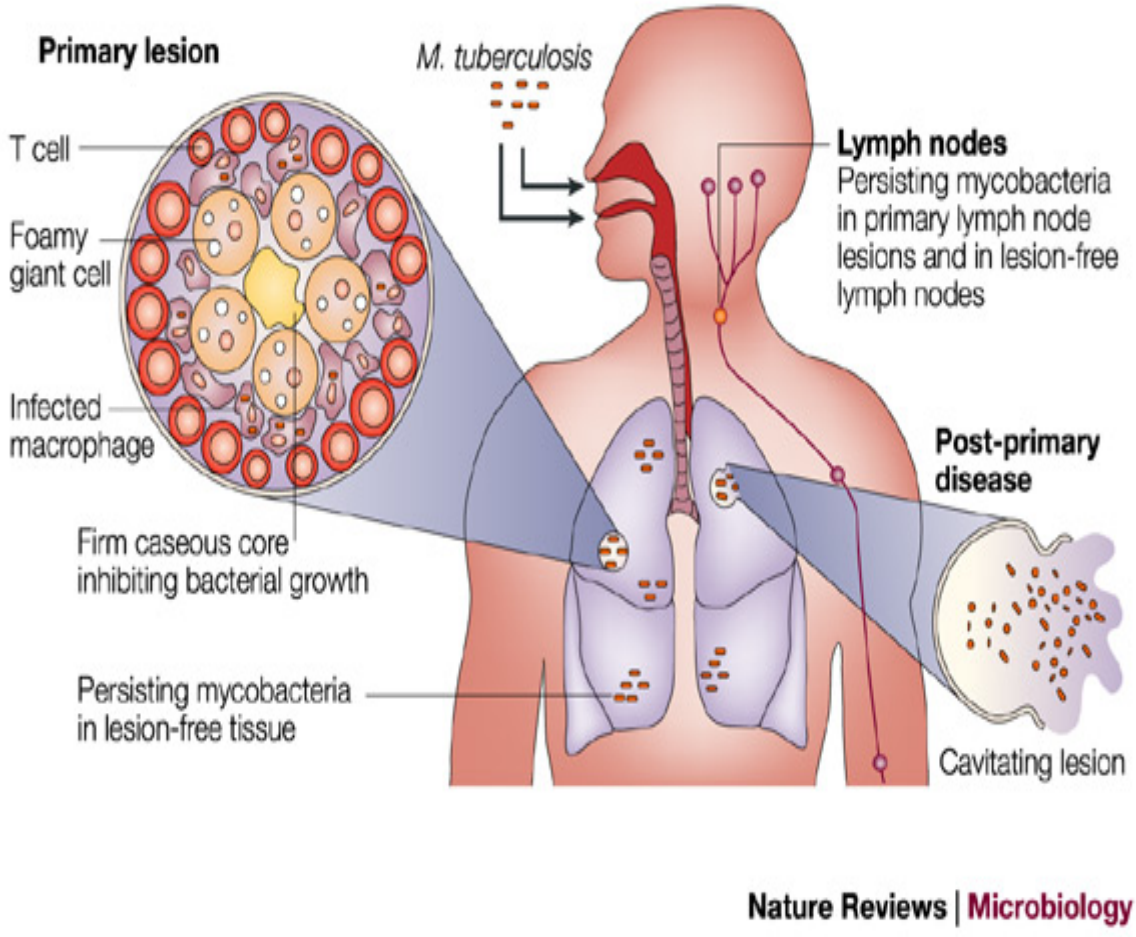
Granulotomatous lesions are important in both regulating the immune response and minimizing tissue damage.

Moreover, T cells help maintain *Mycobacterium* within the granulomas.

The ability to construct *M. tuberculosis* mutants and test individual gene products for specific functions has significantly advanced the understanding of the pathogenesis and virulence factors of *M. tuberculosis*.

Many secreted and exported proteins are known to be important in pathogenesis. Aerolysin is a virulence factor of the pathogenic bacterium Aeromonas hydrophila.

Resistant strains of mycobacterium tuberculosis have developed resistance to more than one TB drug, due to mutations in their genes.



Strain variation

Typing of strains is useful in the investigation of tuberculosis outbreaks, because it gives the investigator evidence for-or-against transmission from person to person.

Consider the situation where person A has tuberculosis and believes he acquired it from person B. If the bacteria isolated from each person belong to different types, then transmission from B to A is definitively disproved; however, if

the bacteria are the same strain, then this supports (but does not definitively prove) the hypothesis that B infected A.

Until the early 2000s, *M. tuberculosis* strains were typed by pulsed field gel electrophoresis (PFGE).

This has now been superseded by variable numbers of tandem repeats (VNTR), which is technically easier to perform and allows

better discrimination between strains.

This method makes use of the presence of repeated DNA sequences within the *M. tuberculosis* genome.

Three generations of VNTR typing for *M. tuberculosis* are noted.

The first scheme, called exact tandem repeat, used only five loci, but the resolution afforded by these five loci was not as good as PFGE. The second scheme, called mycobacterial interspersed repetitive unit, had discrimination as good as PFGE.

The third generation (mycobacterial interspersed repetitive unit - 2) added a further nine loci to bring the total to 24. This provides a degree of resolution greater than PFGE and is currently the standard for typing *M. tuberculosis*.

However, with regard to archaeological remains, additional evidence may be required because of possible contamination from related soil bacteria.

Antibiotic resistance in *M. tuberculosis* typically occurs due to either the accumulation of mutations in the genes targeted by the antibiotic or a change in titration of the drug.

M. tuberculosis is considered to be multidrug-resistant (MDR TB) if it has developed drug resistance to both rifampicin and isoniazid, which are the most important antibiotics used in treatment.

Additionally, extensivley drug-resistant *M. tuberculosis*(XDR TB) is characterized by resistance to both isoniazid and rifampin, plus any fluoroquinolone

and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin).

Evolution

The *M. tuberculosis* complex evolved in Africa and most probably in the Horn of Africa.

The *M.tuberculosis* group has a number of members that include: *Mycobacterium africanum*,

Mycobacterium bovis (Dassie's bacillus),

Mycobacterium caprae,

Mycobacterium microti,

Mycobacterium orygis,

Mycobacterium pinnipedii.

This group may also include the *Mycobacterium canettii* clade.

The *M. canettii* clade — which includes *Mycobacterium prototuberculosis* — is a group of smooth-colony *Mycobacterium* species.

Unlike the established members of the *M. tuberculosis* group, they undergo recombination with other species. The majority of the known strains of this group have been isolated from the Horn of Africa.

The ancestor of *Mycobacterium tuberculosis* appears to be the species *Mycobacterium canettii*, first described in 1969.

The established members of the *M. tuberculosis* complex are all clonal in their spread.

The main human-infecting species have been classified into seven spoligotypes:

- **type 1** contains the East African-Indian (EAI) and some Manu (Indian) strains;
- **type 2** is the Beijing group;
- **type 3** consists of the Central Asian (CAS) strains;
- **type 4** of the Ghana and Haarlem (H/T), Latin America-Mediterranean (LAM) and X strains;
- **types 5** and **6** correspond to *Mycobacterium africanum* and are observed predominantly and at very high frequency in West Africa.
- **type 7** has been isolated from the Horn of Africa. The other species of this complex belong to a number of spoligotypes and do not normally infect humans.

Types 2 and 3 are more closely related to each other than to the other types. Types 5 and 6 are most closely aligned with the species that do not normally infect humans.

Type 3 has been divided into two clades: CAS-Kili (found in Tanzania) and CAS-Delhi (found in India and Saudi Arabia).

The most recent common ancestor of the *M. tuberculosis* complex evolved ~40,000 years ago.

The most recent common ancestor of the EAI and LAM strains has been estimated to be 13,700 and 7,000 years ago, respectively. The Beijing- CAS strains diverged about 17,100 years ago.

All types of the *M. tuberculosis* began their current expansion about 5000 years ago—a period that coincides with the appearance of *M. bovis*.

The Beijing strain appears to have been the most successful with around a 500-fold increase in effective population size (N_e) since its expansion began.

The least successful of the main lineages appears to have been those limited to Africa, where they have undergone an N_e increase of only five-fold.

Since its initial evolution, *M. bovis* has undergone an expansion of N_e of about 65-fold.

Co-evolution with modern humans

There is much evidence to suggest that the different strains of the obligate human pathogen *M. tuberculosis* have co-evolved, migrated, and expanded with their human hosts.

This well-supported theory is consistent with the bacterium's phylogeny and phylogeography. With the global spread of *M. tuberculosis*, studies have examined whether geographically defined human populations are especially susceptible to the transmission of a particular lineage or strain of *M. tuberculosis*.

They have found that even when transmission of *M. tuberculosis* occurs in an urban center outside the region of origin, a human host's region of origin is predictive of which TB strain they carry and that genetically differentiated populations of *M. tuberculosis* do indeed preserve stable associations with host populations from their geographic region.

The fact that all six principle phylogeographic lineages are found in Africa combined with the belief that ancestral Mycobacteria may have impacted early hominids in East Africa as early as three million years ago, once again point to the theory of *M. tuberculosis* originating in Africa and expanding alongside the human migration out of East Africa.

The significant correlation of increased frequency of tuberculosis resistant alleles with the duration of a human population's urban settlement similarly points to an extensive co-evolutionary relationship.

Some of the most compelling data concerning the co-expansion of *M. tuberculosis* with modern humans comes from a study that compared *M.*

tuberculosis phylogeny to human mitochondrial genomes and found impressive similarities in the patterns and geographical locations of branching and divergence events.

The match between *M. tuberculosis* and human mitochondrial phylogenies supports an extended relationship between *M. tuberculosis* and its host, while the clear expansion of this bacterial pathogen during the Neolithic Demographic Transition (~10 000 years ago) suggests that *M. tuberculosis* was able to adapt to changing human populations and that the historical success of this pathogen was driven at least in part by dramatic increases in human host population density.

Genome

The genome of the H37Rv strain was published in 1998. Its size is 4 million base pairs, with 3959 genes; 40% of these genes have had their function characterised, with possible function postulated for another 44%. Within the genome are also six pseudogenes.

The genome contains 250 genes involved in fatty acid metabolism,

with 39 of these involved in the polyketide metabolism generating the waxy coat. Such large numbers of conserved genes show the evolutionary importance of the waxy coat to pathogen survival.

Furthermore, experimental studies have since validated the importance of a lipid metabolism for *M. tuberculosis*, consisting entirely of host-derived lipids such as fats and cholesterol.

Bacteria isolated from the lungs of infected mice were shown to preferentially use fatty acids over carbohydrate substrates.

M. tuberculosis can also grow on the lipid cholesterol as a sole source of carbon, and genes involved in the cholesterol use pathway(s) have been validated as

important during various stages of the infection lifecycle of *M. tuberculosis*, especially during the chronic phase of infection when other nutrients are likely not available.

About 10% of the coding capacity is taken up by the *PE*/*PPE* gene families that encode acidic, glycine-rich proteins.

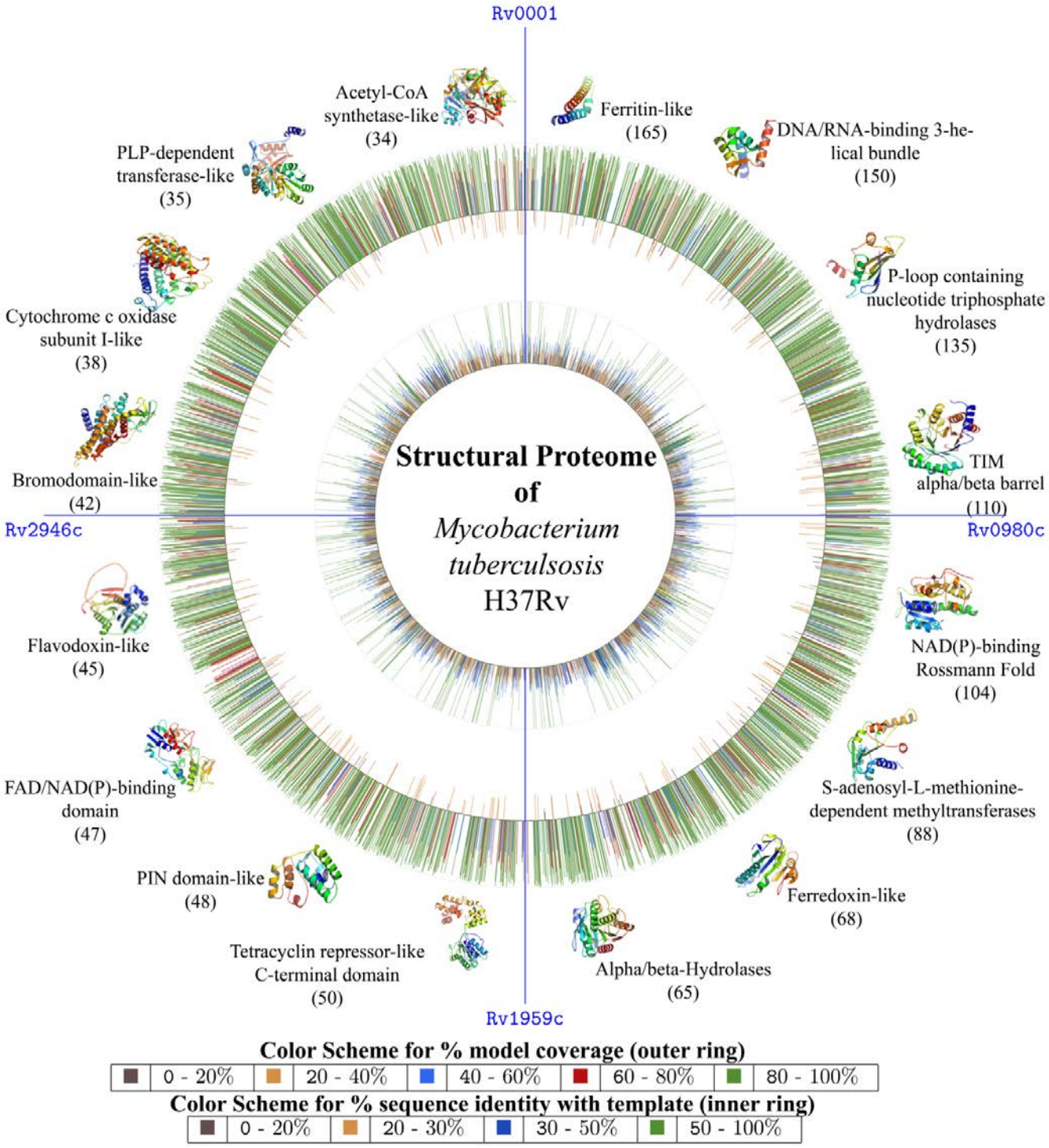
These proteins have a conserved N-terminal motif, deletion of which impairs growth in macrophages and granulomas.

Nine noncoding sRNAs have been characterised in *M. tuberculosis*, with a further 56 predicted in a bioinformatics screen.

In 2013, a study on the genome of several sensitive, ultraresistant, and multiresistant *M. tuberculosis* strains was made to study antibiotic resistance mechanisms.

Results reveal new relationships and drug resistance genes not previously associated and suggest some genes and intergenic regions associated with drug resistance may be involved in the resistance to more than one drug.

Noteworthy is the role of the intergenic regions in the development of this resistance, and most of the genes proposed in this study to be responsible for drug resistance have an essential role in the development of *M. tuberculosis*.



Genetics of Mycobacterium tuberculosis

The nature of the host-pathogen interaction between humans and *Mycobacterium tuberculosis* is considered to have a genetic component. A group of rare disorders called Mendelian Susceptibility to Mycobacterial Diseases

(MSMD) was observed in a subset of individuals with a genetic defect that results in increased susceptibility to Mycobacterial infection.

Early case and twin studies have indicated that genetic component are important in host susceptibility to *Mycobacterial tuberculosis*.

Recent Genome-wide association studies (GWAS) have identified three genetic risk loci, including at positions 11p13 and 18q11.

As is common in GWAS, the variants discovered have moderate effect sizes.

DNA repair

As an intracellular pathogen *M. tuberculosis* is exposed to a variety of DNA damaging assaults, primarily from host-generated antimicrobial toxic radicals.

Exposure to reactive oxygen species and/or reactive nitrogen species causes different types of DNA damage including oxidation, depurination, methylation and deamination that can give rise to single- and double-strand breaks (DSBs).

DnaE2 polymerase is upregulated in *M. tuberculosis* by several DNA damaging agents as well as during infection of mice. Loss of this DNA polymerase reduces the virulence of *M. tuberculosis* in mice.

DnaE2 is an error-prone repair DNA repair polymerase that appears to contribute to *M. tuberculosis* survival during infection.

The two major pathways employed in repair of DSBs are homologous recombinational repair (HR) and non-homologous end joining (NHEJ). Macrophage-internalized *M. tuberculosis* is able to persist if either of these pathways is defective, but is attenuated when both pathways are defective.

This indicates that intracellular exposure of *M. tuberculosis* to reactive oxygen and/or reactive nitrogen species results in the formation of DSBs that are repaired by HR or NHEJ.

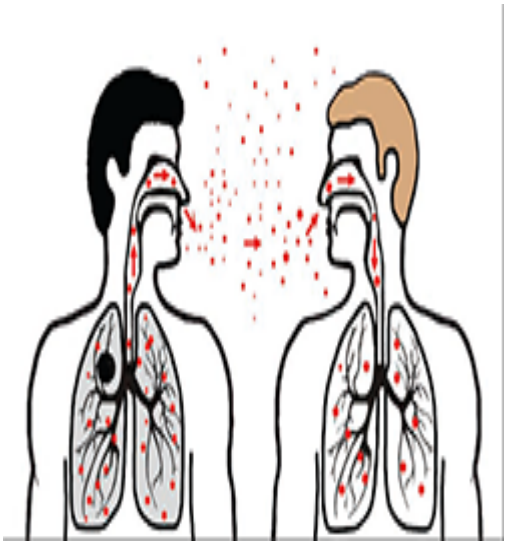
However deficiency of DSB repair does not appear to impair *M. tuberculosis* virulence in animal models.

Basic TB *Facts*

Tuberculosis (TB) is caused by a bacterium called *Mycobacterium tuberculosis*.

The bacteria usually attack the lungs, but TB bacteria can attack any part of the body such as the kidney, spine, and brain. If not treated properly, TB disease can be fatal.

How Tuberculosis Spreads



TB is spread through the air from one person to another. The TB bacteria are put into the air when a person with TB disease of the lungs or throat coughs, sneezes, speaks, or sings.
People nearby may breathe in these bacteria and becomeinfected.

Tuberculosis is NOT spread by

- shaking someone's hand
- sharing food or drink
- touching bed linens or toilet seats
- sharing toothbrushes
- kissing

Latent Tuberculosis Infection and Tuberculosis Disease

Not everyone infected with Tuberculosis bacteria becomes sick. As a result, two Tuberculosis -related conditions exist: latent Tuberculosis infection and Tuberculosis disease.

Latent Tuberculosis Infection

Tuberculosis bacteria can live in the body without making you sick. This is called latent Tuberculosis infection. In most people who breathe in Tuberculosis bacteria and become infected, the body is able to fight the bacteria to stop them from growing.

People with latent Tuberculosis infection do not feel sick and do not have any symptoms.

People with latent Tuberculosis infection are not infectious and cannot spread Tuberculosis bacteria to others.

However, if Tuberculosis bacteria become active in the body and multiply, the person will go from having latent Tuberculosis infection to being sick with Tuberculosis disease.

Tuberculosis Disease

Tuberculosis bacteria become active if the immune system can't stop them from growing. When Tuberculosis bacteria are active (multiplying in your body), this is called Tuberculosis disease.

People with Tuberculosis disease are sick. They may also be able to spread the bacteria to people they spend time with every day.

Many people who have latent Tuberculosis infection never develop Tuberculosis disease.

Some people develop Tuberculosis disease soon after becoming infected (within weeks) before their immune system can fight the Tuberculosis bacteria.

Other people may get sick years later when their immune system becomes weak for another reason.

For people whose immune systems are weak, especially those with HIV infection, the risk of developing Tuberculosis disease is much higher than for people with normal immune systems.

Symptoms of Tuberculosis disease include:

- a bad cough that lasts 3 weeks or longer
- pain in the chest
- coughing up blood or sputum
- weakness or fatigue
- weight loss
- no appetite
- chills
- fever
- sweating at night

Risk Factors

Some people develop Tuberculosis disease soon after becoming infected (within weeks) before their immune system can fight the Tuberculosis bacteria. Other people may get sick years later, when their immune system becomes weak for another reason.

Overall, about 5 to 10% of infected persons who do not receive treatment for latent Tuberculosis infection will develop TB disease at some time in their lives.

For persons whose immune systems are weak, especially those with HIV infection, the risk of developing TB disease is much higher than for persons with normal immune systems.

Generally, persons at high risk for developing Tuberculosis disease fall into two categories:

- Persons who have been recently infected with Tuberculosis bacteria
- Persons with medical conditions that weaken the immune system.

Persons who have been Recently Infected with Tuberculosis Bacteria

This includes:

- Close contacts of a person with infectious TB disease
- Persons who have immigrated from areas of the world with high rates of TB
- Children less than 5 years of age who have a positive TB test
- Groups with high rates of TB transmission, such as homeless persons, injection drug users, and persons with HIV infection
- Persons who work or reside with people who are at high risk for TB in facilities or institutions such as hospitals, homeless shelters, correctional facilities, nursing homes, and residential homes for those with HIV.

Persons with Medical Conditions that Weaken the Immune System

Babies and young children often have weak immune systems. Other people can have weak immune systems, too, especially people with any of these conditions:

- HIV infection (the virus that causes AIDS)
- Substance abuse
- Silicosis
- Diabetes mellitus
- Severe kidney disease
- Low body weight
- Organ transplants

- Head and neck cancer
- Medical treatments such as corticosteroids or organ transplant
- Specialized treatment for rheumatoid arthritis or Crohn's disease

Testing & Diagnosis of Tuberculosis

Tuberculosis is a disease that is spread through the air from one person to another.

There are two kinds of tests that are used to determine if a person has been infected with Tuberculosis bacteria: the tuberculin skin test and Tuberculosis blood tests.

A positive Tuberculosis skin test or Tuberculosis blood test only tells that a person has been infected with Tuberculosis bacteria. It does not tell whether the person has latent Tuberculosis infection (LTBI) or has progressed to Tuberculosis disease.

Other tests, such as a chest x-ray and a sample of sputum, are needed to see whether the person has Tuberculosis disease.

Tuberculin skin test:

The Tuberculosis skin test (also called the Mantoux tuberculin skin test) is performed by injecting a small amount of fluid (called tuberculin) into the skin in the lower part of the arm.

A person given the tuberculin skin test must return within 48 to 72 hours to have a trained health care worker look for a reaction on the arm.

The health care worker will look for a raised, hard area or swelling, and if present, measure its size using a ruler. Redness by itself is not considered part of the reaction. The skin test result depends on the size of the raised, hard area or swelling.

It also depends on the person's risk of being infected with Tuberculosis bacteria and the progression to Tuberculosis disease if infected.

- **Positive skin test:** This means the person's body was infected with Tuberculosis bacteria.

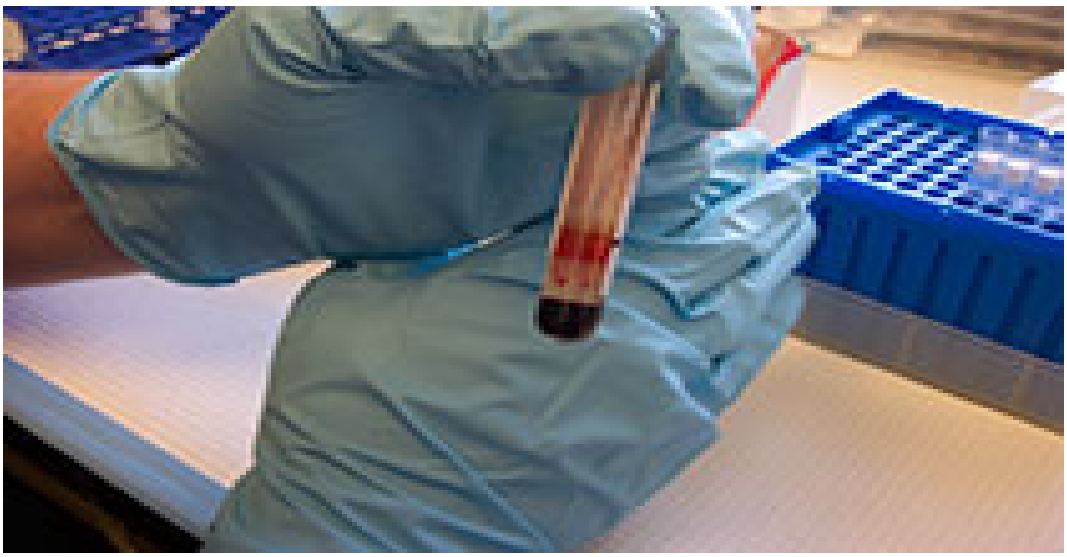
  Additional tests are needed to determine if the person has latent Tuberculosis infection or Tuberculosis disease.

  A health care worker will then provide treatment as needed.
- **Negative skin test:** This means the person's body did not react to the test, and that latent Tuberculosis infection or Tuberculosis disease is not likely.

Tuberculosis blood tests:

Tuberculosis blood tests (also called interferon-gamma release assays or IGRAs) measure how the immune system reacts to the bacteria that cause Tuberculosis.

An IGRA measures how strong a person's immune system reacts to Tuberculosis bacteria by testing the person's blood in a laboratory.



Two IGRAs are approved by the U.S. Food and Drug Administration (FDA) and are available in the United States:

1. QuantiFERON®–TB Gold In-Tube test (QFT-GIT)
2. T-SPOT®.TB test (T-Spot)

- **Positive IGRA:** This means that the person has been infected with Tuberculosis bacteria. Additional tests are needed to determine if the person has latent Tuberculosis infection or Tuberculosis disease.
  A health care worker will then provide treatment as needed.
- **Negative IGRA:** This means that the person's blood did not react to the test and that latent Tuberculosis infection or Tuberculosis disease is not likely.

IGRAs are the preferred method of TB infection testing for the following:

- People who have received bacille Calmette–Guérin (BCG). BCG is a vaccine for Tuberculosis disease.
- People who have a difficult time returning for a second appointment to look for a reaction to the TST.

There is no problem with repeated IGRAs.

Who Should Get Tested for TB

Tuberculosis tests are generally not needed for people with a low risk of infection with Tuberculosis bacteria.

Certain people should be tested for Tuberculosis bacteria because they are more likely to get Tuberculosis disease, including:

- People who have spent time with someone who has Tuberculosis disease
- People with HIV infection or another medical problem that weakens the immune system

- People who have symptoms of Tuberculosis disease (fever, night sweats, cough, and weight loss)
- People from a country where Tuberculosis disease is common (most countries in Latin America, the Caribbean, Africa, Asia, Eastern Europe, and Russia)
- People who live or work somewhere in the United States where Tuberculosis disease is more common (homeless shelters, prison or jails, or some nursing homes)
- People who use illegal drugs.

Testing for Tuberculosis in BCG-Vaccinated Persons

Many people born outside of the United States have been BCG-vaccinated.

People who have had a previous BCG vaccine may receive a Tuberculosis skin test. In some people, BCG may cause a positive skin test when they are not infected with Tuberculosis bacteria.

If a Tuberculosis skin test is positive, additional tests are needed.

IGRAs, unlike the Tuberculosis skin tests, are not affected by prior BCG vaccination and are not expected to give a false-positive result in people who have received BCG.

Choosing a Tuberculosis Test

The person's health care provider should choose which Tuberculosis test to use.

Factors in selecting which test to use include the reason for testing, test availability, and cost.

Generally, it is not recommended to test a person with both a TST and an IGRA.

Diagnosis of Latent Tuberculosis Infection or Tuberculosis Disease

If a person is found to be infected with Tuberculosis bacteria, other tests are needed to see if the person has Tuberculosis disease.

Tuberculosis disease can be diagnosed by medical history, physical examination, chest x-ray, and other laboratory tests.

Tuberculosis disease is treated by taking several drugs as recommended by a health care provider.

If a person does not have Tuberculosis disease, but has Tuberculosis bacteria in the body, then latent Tuberculosis infection is diagnosed.

The decision about treatment for latent Tuberculosis infection will be based on a person's chances of developing Tuberculosis disease.

Infection Control and Prevention

Control program designed to ensure the following:

- prompt detection of infectious patients,
- airborne precautions, and
- treatment of people who have suspected or confirmed Tuberculosis disease.

In order to be effective, the primary emphasis of a Tuberculosis infection control program should be on achieving these three goals.

In all health care settings, particularly those in which people are at high risk for exposure to Tuberculosis, policies and procedures for Tuberculosis control should be developed, reviewed periodically, and evaluated for effectiveness to determine the actions necessary to minimize the risk for transmission of Tuberculosis.

The Tuberculosis infection control program should be based on a three-level hierarchy of control measures and include:

1. Administrative measures
2. Environmental controls
3. Use of respiratory protective equipment

The first and most important level of the hierarchy, administrative measures, impacts the largest number of people.

It is intended primarily to reduce the risk of uninfected people who are exposed to people who have Tuberculosis disease.

The second level of the hierarchy is the use of environmental controls to reduce the amount of Tuberculosis in the air.

The first two control levels of the hierarchy also minimize the number of areas in the health care setting where exposure to Tuberculosis may occur.

The third level of the hierarchy is the use of respiratory protective equipment in situations that pose a high risk of exposure to Tuberculosis. Use of respiratory protection equipment can further reduce the risk for exposure of health care workers.

Tuberculosis Prevention

Preventing Exposure to TB Disease While Traveling Abroad

Travelers should avoid close contact or prolonged time with known Tuberculosis patients in crowded, enclosed environments (for example, clinics, hospitals, prisons, or homeless shelters).

Travelers who will be working in clinics, hospitals, or other health care settings where Tuberculosis patients are likely to be encountered should consult infection control or occupational health experts.

They should ask about administrative and environmental procedures for preventing exposure to Tuberculosis.

Once those procedures are implemented, additional measures could include using personal respiratory protective devices.

Travelers who anticipate possible prolonged exposure to people with Tuberculosis (for example, those who expect to come in contact routinely with clinic, hospital, prison, or homeless shelter populations) should have a tuberculin skin test (TST) or interferon-gamma release assay (IGRA) test before leaving the United States.

If the test reaction is negative, they should have a repeat test 8 to 10 weeks after returning to the United States.

Additionally, annual testing may be recommended for those who anticipate repeated or prolonged exposure or an extended stay over a period of years.

Because people with HIV infection are more likely to have an impaired response to both the TST and IGRA, travelers who are HIV positive should tell their physicians about their HIV infection status.

What to Do If You Have Been Exposed to Tuberculosis

If you think you have been exposed to someone with Tuberculosis disease, contact your health care provider or local health department to see if you should be tested for Tuberculosis.

Be sure to tell the doctor or nurse when you spent time with someone who has Tuberculosis disease.

Preventing Latent Tuberculosis Infection from Progressing to Tuberculosis Disease

Many people who have latent Tuberculosis infection never develop Tuberculosis disease. But some people who have latent Tuberculosis infection are more likely to develop Tuberculosis disease than others.

Those at high risk for developing Tuberculosis disease include:

- People with HIV infection
- People who became infected with Tuberculosis bacteria in the last 2 years
- Babies and young children
- People who inject illegal drugs
- People who are sick with other diseases that weaken the immune system
- Elderly people
- People who were not treated correctly for Tuberculosis in the past.

If you have latent Tuberculosis infection and you are in one of these high-risk groups, you should take medicine to keep from developing Tuberculosis disease. There are several treatment options for latent Tuberculosis infection.

You and your health care provider must decide which treatment is best for you. If you take your medicine as instructed, it can keep you from developing Tuberculosis disease.

Because there are less bacteria, treatment for latent Tuberculosis infection is much easier than treatment for Tuberculosis disease.

A person with Tuberculosis disease has a large amount of Tuberculosis bacteria in the body. Several drugs are needed to treat Tuberculosis disease.

Drug-Resistant Tuberculosis

Drug-resistant Tuberculosis can occur when the drugs used to treat Tuberculosis are misused or mismanaged.

Examples include:

- When people do not complete the full course of treatment;
- When health care providers prescribe the wrong treatment, the wrong dose, or wrong length of time for taking the drugs;
- When the supply of drugs is not always available; or
- When the drugs are of poor quality.

Drug-resistant Tuberculosis is more common in people who:

- Do not take their Tuberculosis drugs regularly
- Do not take all of their Tuberculosis drugs
- Develop Tuberculosis disease again, after being treated for Tuberculosis disease in the past
- Come from areas of the world where drug-resistant Tuberculosis is common
- Have spent time with someone known to have drug-resistant Tuberculosis disease.

Drug-resistant Tuberculosis is spread the same way that drug susceptible Tuberculosis is spread. Tuberculosis is spread through the air from one person to another.

The Tuberculosis bacteria are put into the air when a person with Tuberculosis disease of the lungs or throat coughs, sneezes, speaks, or sings. People nearby may breathe in these bacteria and become infected.

Multidrug-Resistant Tuberculosis (MDR Tuberculosis)

Multidrug-resistant Tuberculosis (MDR Tuberculosis) is caused by an organism that is resistant to at least isoniazid and rifampin, the two most potent Tuberculosis drugs. These drugs are used to treat all persons with Tuberculosis disease.

- Multidrug-Resistant Tuberculosis (Fact sheet)

Extensively Drug-resistant Tuberculosis (XDR Tuberculosis)

Extensively drug-resistant Tuberculosis (XDR Tuberculosis) is a rare type of MDR Tuberculosis that is resistant to isoniazid and rifampin, plus any fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin).

Because XDR Tuberculosis is resistant to the most potent Tuberculosis drugs, patients are left with treatment options that are much less effective.

XDR Tuberculosis is of special concern for people with HIV infection or other conditions that can weaken the immune system. These people are more likely to develop Tuberculosis disease once they are infected, and also have a higher risk of death once they develop Tuberculosis.

- Extensively Drug-Resistant Tuberculosis

Preventing Drug-Resistant Tuberculosis

The most important way to prevent the spread of drug-resistant Tuberculosis is to take all Tuberculosis drugs exactly as prescribed by the health care provider. No doses should be missed and treatment should not be stopped early.

People receiving treatment for Tuberculosis disease should tell their health care provider if they are having trouble taking the drugs.

Health care providers can help prevent drug-resistant Tuberculosis by quickly diagnosing cases, following recommended treatment guidelines, monitoring patients' response to treatment, and making sure therapy is completed.

Another way to prevent getting drug-resistant Tuberculosis is to avoid exposure to known drug-resistant Tuberculosis patients in closed or crowded places such as hospitals, prisons, or homeless shelters.

People who work in hospitals or health-care settings where Tuberculosis patients are likely to be seen sh

ould consult infection control or occupational health experts.

Treatment

Treatment for Latent Tuberculosis Infection

People with latent TB infection have Tuberculosis bacteria in their bodies, but they are not sick because the bacteria are not active. People with latent Tuberculosis infection do not have symptoms, and they cannot spread Tuberculosis bacteria to others.

However, if Tuberculosis bacteria become active in the body and multiply, the person will go from having latent Tuberculosis infection to being sick with Tuberculosis disease.

For this reason, people with latent Tuberculosis infection are often prescribed treatment to prevent them from developing Tuberculosis disease. Treatment of latent Tuberculosis infection is essential for controlling and eliminating.

Because there are less bacteria in a person with latent Tuberculosis infection, treatment is much easier. Four regimens are approved for the treatment of latent infection.

The medications used to treat latent infection include:

- isoniazid (INH)

- rifampin (RIF)
- rifapentine (RPT)

Certain groups of people (such as people with weakened immune systems) are at very high risk of developing TB disease once infected with Tuberculosis bacteria. Every effort should be made to begin appropriate treatment and to ensure completion of the entire course of treatment for latent Tuberculosis infection.

Treatment for Tuberculosis Disease

Tuberculosis bacteria become active (multiplying in the body) if the immune system can't stop them from growing.

When Tuberculosis bacteria are active, this is called TB disease. Tuberculosis disease will make a person sick.

People with Tuberculosis disease may spread the bacteria to people with whom they spend many hours.

Tuberculosis disease can be treated by taking several drugs for 6 to 9 months. There are 10 drugs currently approved by the U.S. Food and Drug Administration (FDA) for treating Tuberculosis.

Of the approved drugs, the first-line anti- Tuberculosis agents that form the core of treatment regimens include:

- isoniazid (INH)
- rifampin (RIF)
- ethambutol (EMB)
- pyrazinamide (PZA)

Regimens for treating Tuberculosis disease have an initial phase of 2 months, followed by a choice of several options for the *continuation phase* of either 4 or 7 months (total of 6 to 9 months for treatment).

Learn more about the continuation phase of treatment.

It is very important that people who have Tuberculosis disease finish the medicine, taking the drugs exactly as prescribed. If they stop taking the drugs too soon, they can become sick again; if they do not take the drugs correctly, the

Tuberculosis bacteria that are still alive may become resistant to those drugs. Tuberculosis that is resistant to drugs is harder and more expensive to treat.

Tuberculosis and HIV Coinfection

Even though fewer people in the United States have tuberculosis (TB), it remains a serious threat, especially for people living with HIV. People living with HIV are more likely than others to become sick with Tuberculosis.

Worldwide, Tuberculosis is one of the leading causes of death among people living with HIV.

Without treatment, as with other opportunistic infections, HIV and Tuberculosis can work together to shorten lifespan.

- Someone with untreated latent TB infection and HIV infection is much more likely to develop TB disease during his or her lifetime than someone without HIV infection.
- Among people with latent Tuberculosis infection, HIV infection is the strongest known risk factor for progressing to Tuberculosis disease.
- A person who has both HIV infection and Tuberculosis disease has an AIDS-defining condition.

People infected with HIV who also have either latent Tuberculosis infection or Tuberculosis disease can be effectively treated. The first step is to ensure that people living with HIV are tested for Tuberculosis infection.

If found to have Tuberculosis infection, further tests are needed to rule out TB disease. The next step is to start treatment for latent Tuberculosis infection or Tuberculosis disease based on test results.

Treatment

Untreated latent Tuberculosis infection can quickly progress to Tuberculosis disease in people living with HIV since the immune system is already weakened. And without treatment, Tuberculosis disease can progress from sickness to death.

Fortunately, there are a number of treatment options for people living with HIV who also have either latent Tuberculosis infection or Tuberculosis disease.

Consult with your state or local health department for treatment options.

Vaccine and Immunizations

Bacille Calmette-Guérin (BCG) is a vaccine for tuberculosis (TB) disease. This vaccine is not widely used in the United States, but it is often given to infants and small children in other countries where TB is common.

BCG does not always protect people from getting Tuberculosis.

BCG Recommendations

In the United States, BCG should be considered for only very select people who meet specific criteria and in consultation with a Tuberculosis expert.

Health care providers who are considering BCG vaccination for their patients are encouraged to discuss this intervention with the TB control program in their area.

Children

BCG vaccination should only be considered for children who have a negative TB skin test and who are continually exposed, and cannot be separated from adults who

- Are untreated or ineffectively treated for TB disease, and the child cannot be given long-term primary preventive treatment for Tuberculosis infection; or
- Have Tuberculosis disease caused by strains resistant to isoniazid and rifampin.

Health Care Workers

BCG vaccination of health care workers should be considered on an individual basis in settings in which

- A high percentage of Tuberculosis patients are infected with Tuberculosis strains resistant to both isoniazid and rifampin;
- There is ongoing transmission of drug-resistant Tuberculosis strains to health care workers and subsequent infection is likely; or
- Comprehensive TB infection-control precautions have been implemented, but have not been successful.

Health care workers considered for BCG vaccination should be counseled regarding the risks and benefits associated with both BCG vaccination and treatment of latent Tuberculosis infection.

Tuberculosis in Specific Populations

Tuberculosis in the African–American Community

Disparities in tuberculosis (TB) persist among members of racial and ethnic minority populations. In 2010, the majority (84%) of all reported Tuberculosis cases in the United States (US) occurred in racial and ethnic minorities.

Black, non-Hispanic persons, have a disproportionate share of Tuberculosis in the US.

In 2010, Tuberculosis was reported in 2,652 black, non-Hispanic persons, 24% of all persons reported with Tuberculosis nationally. Also, in 2010, the rate of

Tuberculosis in black, non-Hispanic persons was 7.0 cases per 100,000 population, which is approximately 7 times higher than the rate of Tuberculosis in white, non-Hispanic persons (0.9 cases per 100,000 population).

The proportion of Tuberculosis in black, non-Hispanic persons, is even greater if only US-born (African–American) persons reported with TB are examined. In 2010, among US-born persons reported with Tuberculosis, 40% were African Americans (black, non-Hispanic).

Although rates of Tuberculosis in both blacks and whites have declined substantially over the past decade, the disparity remains.

We must better target our efforts to prevent and control TB in this population. Addressing the Tuberculosis disparity among African Americans and other US-born racial/ethnic groups is an important priority.

Children

Tuberculosis disease in children under 15 years of age (also called pediatric tuberculosis) is a public health problem of special significance because it is a marker for recent transmission of Tuberculosis.

Also of special significance, infants and young children are more likely than older children and adults to develop life-threatening forms of Tuberculosis disease (e.g., disseminated Tuberculosis, Tuberculosis meningitis).

Among children, the greatest numbers of Tuberculosis cases are seen in children less than 5 years of age, and in adolescents older than 10 years of age.

If Tuberculosis bacteria become active in the body and multiply, the person will get sick with Tuberculosis disease.

Persons with Tuberculosis disease:

- Usually have a skin test or blood test indicating Tuberculosis infection;

- Are sick from Tuberculosis bacteria that are active (meaning that they are multiplying and destroying tissue in their body);
- Usually have symptoms of Tuberculosis disease; and
- Must be given medicine to treat Tuberculosis disease.

Once infected with Tuberculosis bacteria, children are more likely to get sick with Tuberculosis disease and to get sick more quickly than adults.

In comparison to children, Tuberculosis disease in adults is usually due to past Tuberculosis infection that becomes active years later, when a person's immune system becomes weak for some reason (e.g., HIV infection, diabetes).

Confirming the diagnosis of TB disease in children with a laboratory test can be challenging. This is because:

- It is difficult to collect sputum specimens from infants and young children;
- The laboratory tests used to find Tuberculosis in sputum are less likely to have a positive result in children; this is due to the fact that children are more likely to have Tuberculosis disease caused by a smaller number of bacteria (paucibacillary disease).

For these reasons, the diagnosis of Tuberculosis disease in children is often made without laboratory confirmation and instead based on combination of the following factors:

- Clinical signs and symptoms typically associated with Tuberculosis disease,
- Positive tuberculin skin test (TST) or positive Tuberculosis blood test (IGRA),
- Chest x-ray that has patterns typically associated with Tuberculosis disease,
- History of contact with a person with infectious Tuberculosis disease.

Testing for Tuberculosis in Children

In the absence of symptoms, usually the only sign of Tuberculosis infection is a positive reaction to the Tuberculosis skin test or Tuberculosis blood test. Tuberculosis skin testing is considered safe in children, and is preferred over Tuberculosis blood tests for children less than 5 years of age.

All children with a positive test for Tuberculosis infection, symptoms of Tuberculosis, or a history of contact with a person with infectious Tuberculosis disease should undergo a medical evaluation. Medical evaluations for

Tuberculosis disease include a chest x-ray and physical examination to exclude Tuberculosis disease, and must be done before beginning treatment for latent Tuberculosis infection.

Signs and Symptoms of Tuberculosis Disease in Children

Signs and symptoms of Tuberculosis disease in children include:

- Cough;
- Feelings of sickness or weakness, lethargy, and/or reduced playfulness;
- Weight loss or failure to thrive;
- Fever; and/or
- Night sweats.

The most common form of Tuberculosis disease occurs in the lungs, but Tuberculosis disease can affect other parts of the body as well. Symptoms of Tuberculosis disease in other parts of the body depend on the area affected.

Infants, young children, and immunocompromised children (e.g., children with HIV) are at the highest risk of developing the most severe forms of Tuberculosis such as Tuberculosis meningitis or disseminated Tuberculosis disease.

Treatment

A pediatric Tuberculosis expert should be involved in the treatment of Tuberculosis in children and in the management of infants, young children, and immunocompromised children who have been exposed to someone with infectious Tuberculosis disease.

It is very important that children or anyone being treated for latent Tuberculosis infection or Tuberculosis disease finish the medicine and take the drugs exactly as instructed.

Latent Tuberculosis Infection

Treatment is recommended for children with latent Tuberculosis infection to prevent them from developing Tuberculosis disease.

Infants, young children, and immunocompromised children with latent Tuberculosis infection or children in close contact with someone with infectious Tuberculosis disease, require special consideration because they are at increased risk for getting Tuberculosis disease.

Consultation with a pediatric Tuberculosis expert is recommended before treatment begins. Isoniazid is the anti- Tuberculosis medicine that is most commonly used for treatment of latent Tuberculosis infection.

In children, the recommended length of treatment with isoniazid is 9 months.

Tuberculosis Disease

Tuberculosis disease is treated by taking several anti-TB medicines for 6 to 9 months. It is important to note that if a child stops taking the drugs before

completion, the child can become sick again. If drugs are not taken correctly, the bacteria that are still alive may become resistant to those drugs.

Tuberculosis that is resistant to drugs is harder and more expensive to treat, and treatment lasts much longer (up to 18 to 24 months).

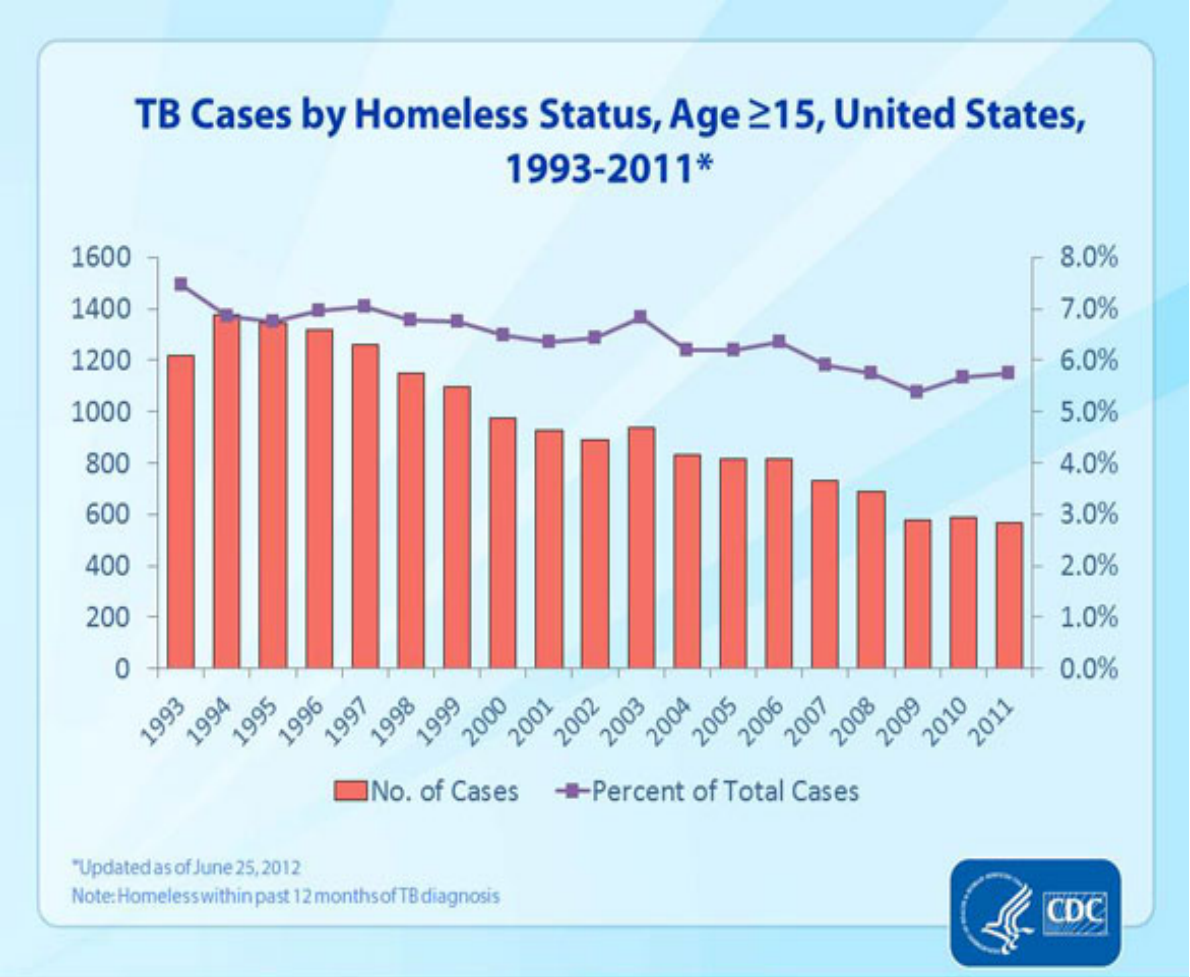
Tuberculosis in the Homeless Population

Tuberculosis in the homeless population is a public health concern. While the reported number of Tuberculosis cases in the United States decreased slightly in 2011, a disproportionate number of Tuberculosis cases still occur among high-risk populations, including people experiencing homelessness.

In the United States, 1% of the population experiences homelessness in a given year, but more than 5% of people with Tuberculosis reported being homeless within the year prior to diagnosis.

These findings are not surprising, as people experiencing homelessness have a high occurrence of conditions that increase the risk of Tuberculosis, including substance abuse, HIV infection, and congregation in crowded shelters. This combination of conditions is favorable for spreading Tuberculosis.

In addition, people who are homeless often lack ready access to the medical care required to make an early diagnosis of Tuberculosis.



International Travelers

In many countries, Tuberculosis is much more common than in the United States. Tuberculosis is a serious international public health problem. Although multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB are occurring globally, they are still rare.

HIV-infected travelers are at greatest risk if they come in contact with a person with MDR or XDR TB.

All travelers should avoid high risk settings where there are no infection control measures in place.

Documented places where transmission has occurred include crowded hospitals, prisons, homeless shelters, and other settings where susceptible persons come in contact with persons with Tuberculosis disease.

Air travel itself carries a relatively low risk of infection with Tuberculosis of any kind.

- Tuberculosis Information for International Travelers (fact sheet)

Pregnancy

While dealing with a tuberculosis (TB) diagnosis in pregnancy is not easy, there is a greater risk to the pregnant woman and her baby if Tuberculosis disease is not treated.

Babies born to women with untreated Tuberculosis disease may have lower birth weight than those babies born to women without Tuberculosis. Rarely, a baby may be born with Tuberculosis.

Pregnant women who are diagnosed with Tuberculosis disease should start treatment as soon as Tuberculosis is detected.

Although the Tuberculosis drugs used in treatment cross the placenta, these drugs do not appear to have harmful effects on the baby.

TB skin testing is considered both valid and safe throughout pregnancy.

TB blood tests also are safe to use during pregnancy, but have not been evaluated for diagnosing Tuberculosis infection in pregnant women.

Other tests are needed to show if a person has Tuberculosis disease.

Mycobacterium Leprae

Taxonomy

| Domain = Bacteria | Phylum = Actinobacteria | Class = Actinobacteridae | Order = Actinomycetales | Suborder = Corynebacterineae | Family = Mycobacteriaceae | Genus =Mycobacterium | Species = M. leprae |

Description

Mycobacterium leprae is a microaerophilic, acid-fast bacillus which causes leprosy.

Since *Mycobacterium leprae* cannot easily be cultured in the lab, much is unknown about the infectious dose, incubation, and transmission of the disease. The infection is thought to be spread through the skin and nasal mucosa.

Humans and armadillos are the only known carriers of the disease, though there is some speculation about the possible role of insects in transmission.

Mycobacterium leprae colonizes the Schwann cells of the peripheral nervous system and can also live and grow within macrophages as a way to evade the host immune system.

The bacteria express many virulence factors that allow for invasion of the nerve cells and nutritional access. The symptoms of leprosy are associated with the degree of host immune system response to the infection, so the disease is classified according to these varied responses.

Polar tuberculoid (TT) leprosy results from the immune system trying to kill the pathogen.

This is in contrast to lepromatous (LL) which is marked by no host resistance and thus direct killing of self-cells through the immune response.

Borderline categories fall between the two polar forms of the disease. Reactions can then be divided into two types based on clinical manifestations. Biopsy from a lesion revealing acid-fast bacilli is necessary for diagnosis.

The standard treatment for leprosy is a Multi-drug Treatment (MDT) that consists of steroids and antibiotics.

Through prevention steps and treatment, the World Health Organization is attempting to eradicate the disease, and has so far succeeded in decreasing the total number of cases.

However, the number of new cases each year remains the same despite some positive results from the *M. bovis* BCG vaccine.

Due to the intracellular lifestyle of the bacteria, *Mycobacterium leprae* infection is associated with symptoms that are the result of the immune system attacking host cells.

The bacteria elicit an immune response on nerve cells and other immune cells, causing inflammation and neuropathy.

Mycobacterium leprae, also known as **Hansen's coccus spirilly**, mostly found in warm tropical countries, is a Gram-positive bacterium that causes leprosy (Hansen's disease). It is an intracellular, pleomorphic, acid-fast, pathogenic bacterium. *M. leprae* is an aerobic bacillus (rod-shaped) surrounded by the characteristic waxy coating unique to mycobacteria.

In size and shape, it closely resembles *Mycobacterium tuberculosis*.

Due to its thick waxy coating, *M. leprae* stains with a carbol fuchsin rather, than with the traditional Gram stain. The culture takes several weeks to mature.

Optical microscopy shows *M. leprae* in clumps, rounded masses, or in groups of bacilli side by side, and ranging from 1–8 μm in length and 0.2–0.5 μm in diameter.

It was discovered in 1873 by the Norwegian physician Gerhard Armauer Hansen, who was searching for the bacteria in the skin nodules of patients with leprosy.

It was the first bacterium to be identified as causing disease in humans. Filippo Pacini (1812-83) published a paper in 1854 entitled, "Microscopical observations and pathological deductions on cholera" in which he identified Vibrio cholera, but Pacini's work was completely ignored by the scientific community.

The organism has never been successfully grown on an artificial cell culture medium. Instead, it has been grown in mouse foot pads and more recently in nine-banded armadillos because they, like humans, are susceptible to leprosy.

This can be used as a diagnostic test for the presence of bacilli in body lesions of suspected leprosy patients.

The difficulty in culturing the organism appears to be because it is an obligate intracellular parasite that lacks many necessary genes for independent survival.

The complex and unique cell wall that makes members of the *Mycobacterium* genus difficult to destroy is apparently also the reason for the extremely slow replication rate.

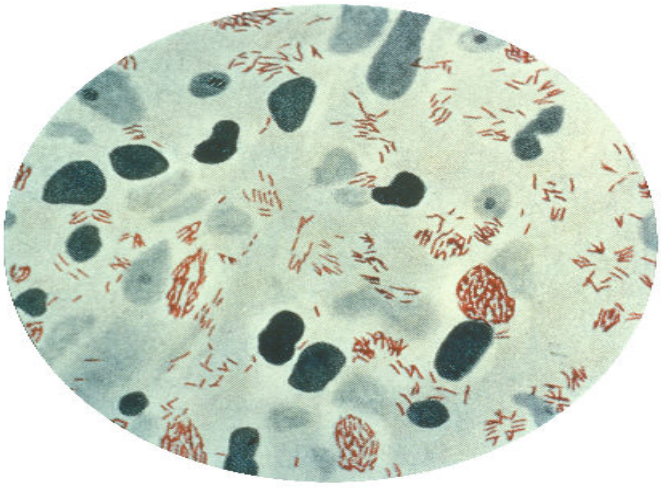
Virulence factors include a waxy exterior coating, formed by the production of mycolic acids unique to *Mycobacterium*.

M. leprae was sensitive to dapsone (diaminodiphenylsulfone, the first effective treatment which was discovered for leprosy in the 1940s), but resistance against this antibiotic has developed over time.

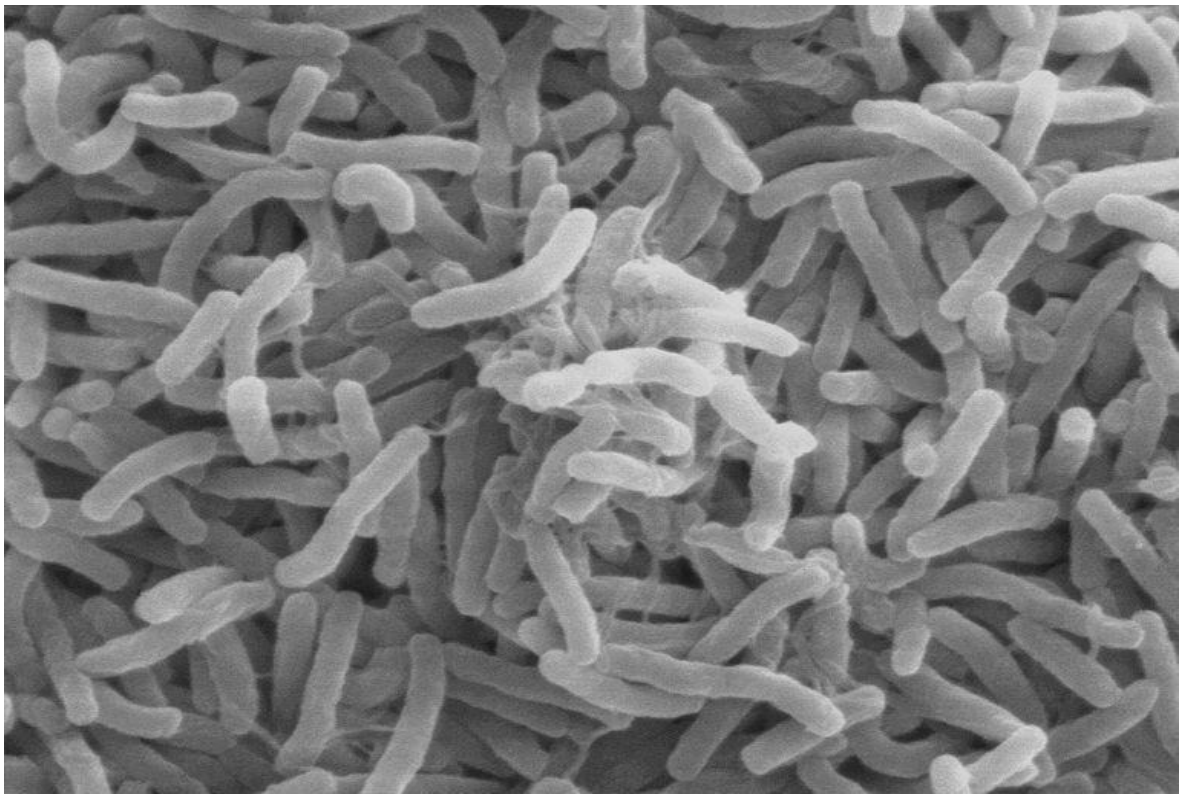
Therapy with dapsone alone is now strongly contraindicated. Currently, a multidrug treatment (MDT) is recommended by the World Health Organization, including dapsone, rifampicin and clofazimine.

In patients receiving the MDT, a high proportion of the bacilli die within a short amount of time without immediate relief of symptoms.

This suggests many symptoms of leprosy must bedue in part to the presence of dead cells.



Stain by Ziehl - Nilssen



Mycobacterium leprae underelectron microscope

Cultivation. It has not so far been possible to cultivate lepra bacilli either in bacteriological media or in tissue culture.

Toxin production. The organisms have not been shown to produce a toxin. They evidently produce allergic substances.

Antigenic structure and classification have not been worked out.

Fermentative properties have been insufficiently studied. This research has been handicapped by failure to solve the problem of cultivation of M. *leprae* on nutrient media.

Resistance. Lepra bacilli have been found to remain viable in warm humid environment for 9-16 days and in moist soil for 46 days.

They survive exposure to direct sunlight for 2 hours and ultraviolet light for 30 minutes.

Pathogenesis and diseases in man. Leprosy is an exclusively human disease and the only source of infection is the patient.

Leprosy is not highly communicable. The disease develops in only about 5 % of spouses living with leprosy patients. However, contacts of patients show a high rate of sensitization to *M. leprae* by lymphocyte transformation tests.

The incubation period is very long and averages 2-5 years. It has been estimated to vary from a few months to as long as 30 years.

Immunity. A high degree of innate immunity against lepra bacilli seems to exist in human beings so that only a minority of those infected develop clinical disease.

Infection with lepra bacilli induces both humoral and cellular immune responses. Humoral antibodies are without deleterious effect on the bacilli, while the cellular immune mechanisms are capable of destroying them.

Treatment. It is used rifampicin, clofazimine.

Genetics

Several genes have been associated with a susceptibility to leprosy. Many people's immune systems are able to eliminate leprosy during the early infection stage before severe symptoms develop.

Research suggests a defect in cell-mediated immunity causes susceptibility to leprosy.

The region of DNA responsible for this variability is also involved in Parkinson's disease, giving rise to current speculation that the two disorders may be linked in some way at the biochemical level.

Some evidence indicates not all people who are infected with *M. leprae* develop leprosy, and genetic factors have long been thought to play a role, due to the observation of clustering of leprosy around certain families, and the failure to understand why certain individuals develop lepromatous leprosy while others develop other types of leprosy.

Name	Locus	OMIM	**Gene**
LPRS1	10p13	609888	
LPRS2	6q25	607572	PARK2, PACRG
LPRS3	4q32	246300	TLR2
LPRS4	6p21.3	610988	LTA
LPRS5	4p14	613223	TLR1
LPRS6	13q14.11	613407	

Pathogenesis

Transmission

Although much about the transmission of *Mycobacterium leprae* is unknown, prolonged contact with an infected person increases an individual's chance of becoming infected.

Armadillos can harbor the bacteria, but are not seen as a threat to human contraction of the disease.

In addition, insects could be possible carriers of *Mycobacterium leprae* but this is unclear In humans, the bacteria is thought to be passed through skin and nasal mucosa.

One study has demonstrated that large numbers of the bacteria can be found on the skin of infected persons, providing a possible means of transmission.

Mycobacterium leprae could also be passed through nasal mucosa like the closely related *Mycobacterium tuberculosis*.

Transmission of leprosy occurs during close contact with those who are infected. Transmission is believed to be by nasal droplets.

Leprosy is not known to be either sexually transmitted or highly infectious. People are no longer infectious after as little as two weeks of treatment.

Leprosy may also be transmitted to humans by armadillos and may be present in three species of non-human primates.

Two exit routes of *M. leprae* from the human body often described are the skin and the nasal mucosa, although their relative importance is not clear.

Lepromatous cases show large numbers of organisms deep in the dermis, but whether they reach the skin surface in sufficient numbers is doubtful.

The skin and the upper respiratory tract are most likely entry route. While older research dealt with the skin route, recent research has increasingly favored the respiratory route.

Experimental transmission of leprosy through aerosols containing *M. leprae* in immunosuppressed mice was accomplished, suggesting a similar possibility in humans.

Infectious dose, incubation, and colonization

Mycobacterium leprae is not easily cultured in the lab, which can hinder studies of infectious dose and incubation, however some sources provide estimates for these categories.

With a doubling time of 14 days, *Mycobacterium leprae* has the longest doubling time of any studied bacteria.

The World Health Organization states that *Mycobacterium leprae* has an incubation period of an average 5 years.

Humans and armadillos are currently the only known reservoirs of the bacteria, with infected humans accounting for up to 7 billion organisms per gram of tissue.

Mycobacterium leprae mostly lives in the extremities and facial region within macrophages and Schwann cells of the peripheral nervous system.

Mycobacterium leprae is thought to have originated in East Africa and spread across the globe through human migratory trends, reaching the Western world within the last 500 years.

In 2012, the World Health Organization recorded a prevalence of approximately 180,000 cases.

Through eradication efforts, the total number of cases worldwide has decreased, yet the number of new cases each year has remained consistent.

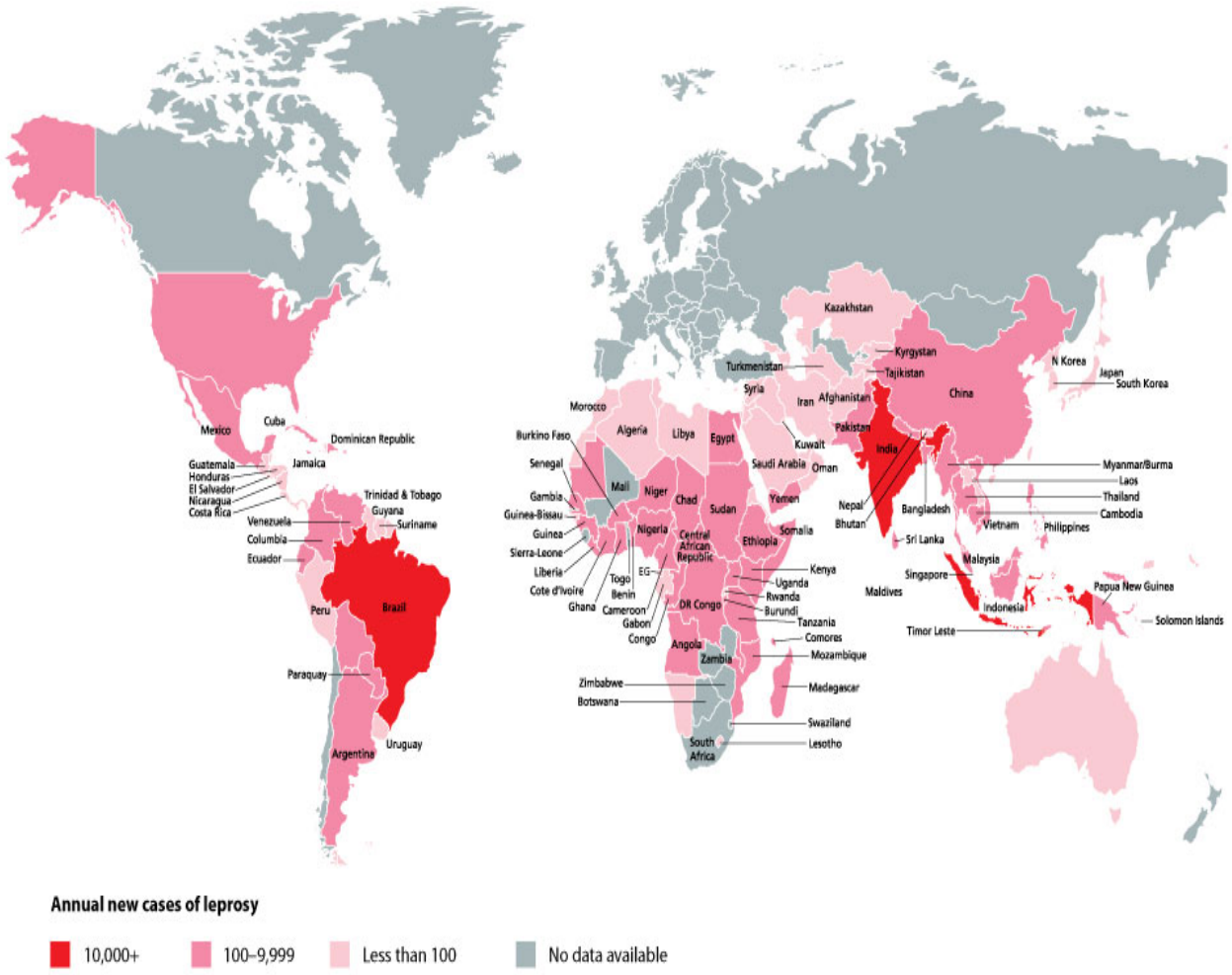
Mortality is difficult to measure with leprosy, as the infection is not the immediate cause of death in many cases.

Around the world, as many as 2 million people are permanently disabled as a result of Hansen's disease.

You may be at risk for the disease if you:

- live in a country where the disease is widespread. Such countries include:
  - Angola
  - Brazil
  - CentralAfrican Republic
  - Democratic Republic of Congo
  - Federated States of Micronesia
  - India
  - Kiribati
  - Madagascar
  - Mozambique
  - Nepa
  - Republic of Marshall Islands
  - United Republic of Tanzania

Epidemiology



Map of new leprosy cases annually

- are in prolonged close contact with people who have untreated Hansen's disease. If they have not been treated, you could be exposed to the bacteria that cause Hansen's disease. As soon as patients start treatment, however, they are no longer able to spread the disease.

Most adults around the world, however, might face no risk at all. That's because evidence shows that 95% of all adults are naturally unable to get the disease, even if they're exposed to the bacteria that causes it.

Date: _____

Protocol of the practical classe

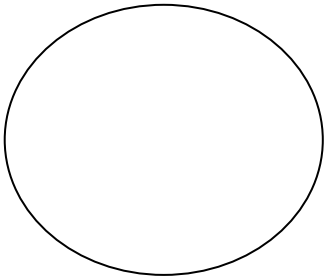
Theme: **Laboratory diagnostics of tuberculosis.**

Questions for the learning.

1. **Characteristic of mycobacteria species, pathogenic ones.**
2. **Causative agents of tuberculosis: species, main properties.**
3. **Pathogenesis, clinical developments of diseases.**
4. **Features of antituberculosis immunity.**
5. **Laboratory diagnostic.**
6. **Treatment and preventive measures of tuberculosis. BCG vaccine, terms, technique of vaccination and revaccination.**

1. Name the taxonomy and draw the morphology of causative agents of tuberculosis.

Causative agent	Tuberculosis
Family	
Genus	
Species	



Methods of staining

_____

Mycobacterium tuberculosis

2. Name the main toxins produced by:

M.tuberculosis

_____

_____

3. Name nutrient media which are used for cultivation and identification of causative agents of tuberculosis.

_____

_____

_____

4. **Name the main parts of epidemic process during diseases caused by causative agents:**

	Tuberculosis
Source of infection	
Mechanism of transmission	

5. Fill in the table.

	Material for investigation	**Laboratory diagnostics**	**Treatment, prevention**
Tuberculosis			

1. In consumptive patient's anamnesis was an open pulmonary form of disease. Which method of staining is the best to use at researching of sputum to find the agent?

A. Method of Romanowsky-Giemsa

B. Method of Neisser

C. Method of Ziehl-Neelsen

D. Method of Burry-Gins

E. Method of Gram

2. Transmission of the causative agent Mycobacterium tuberculosis between humans is primarily through:

A. Sexuel contact

B. Respiratory droplets

C. Contaminated foods

D. Soil (environment)

E. Contaminated blood products

3. The early stage of Mycobacterium tuberculosis infection is characterized by the formation of which of the following lesions in the lungs?

A. Granulomatous

B. Exudative

C. Tubercle

D. Induration

E. Eschar

4. An allergic Manitou test was negative. What does such result of the test testify to?

A. To absence of antitoxic immunity to tuberculosis

B. To presence of cellular immunity to tuberculosis

C. To absence of antibodies to tuberculosis

D. To presence of antibodies to tubercular bacteria

E. To absence of cellular immunity to tuberculosis

5. The patient's sputum was received to bacteriological laboratory for investigation because the doctor has suspected pulmonary tuberculosis. The bacteriologist used the differential- diagnostic nutrient medium for vealing of tubercular bacillus. Name the medium.

A. Blood agar

B. Lowenstein-Jensen medium

C. Wilson-Blair medium

D. Moncur's medium

E. Bordet-Gengou medium

6. Doctor diagnosed that the patient has tuberculosis. What microorganism causes this infection?

A. Shigella

B. Salmonella

C. Mycobacterium

D. Bordetella

E. Escherichia

7. Children are vaccinated against tuberculosis. What kind of vaccine is used for it?

A. Live

B. Anatoxin

C. Chemical

D. Recombinated

E. Killed

8. The epidemic rise of tuberculosis is registered in the city. What material is used in laboratory diagnostics of tuberculosis?

A. Urine

B. Saliva

C. Sputum

D. Feces

E. Nasopharynx wash off

9. Each of the following statements concerning Mycobacterium tuberculosis is correct EXCEPT:

A. It has a large amount of mycolic acid in the cell wall

B. It appears as a red rod in Gram-stained specimens

C. It appears as a red rod in acid-fast staining

D. It can be detected by light microscopy

E. After being stained with carbolfucshin it resists decolorization with acid

10. BCG vaccine contains:

A. Capsular polysaccharide

B. Live attenuated M. bovis

C. Toxoid

D. Live attenuated M. tuberculosis

E. Whole killed M. tuberculosis

12. A patient skin test with purified protein derivative (PPD) to determine previous exposure to Mycobacterium tuberculosis develops induration at the skin test site 48 hours later. Histologically, the reaction site would MOST probably show:

A. eosinophils

B. neutrophils

C. B cells

D. NK cells

E. helper T cells and macrophages

13. Mycobacteria resist intracellular killing due to which cell wall constituent?

A. mycolic acids (wax D)

B. lipopolysaccharide

C. lipoteichoic acid

D. peptidoglycan

E. periplasmic enzymes

14. Which of the following statements characterizes Mycobacteria tuberculosis better?

A. M. tuberculosis cases strong hypersensitivity reaction type IV in infected patients

B. M. tuberculosis produces highly potent exotoxin

C. M. tuberculosis induces production of protective antibodies in infected patients

D. M. tuberculosis can be effectively killed by macrophages

E. M. tuberculosis grow fast on ordinary media

15. Each of the following statements concerning Mycobacterium tuberculosis is correct EXCEPT:

A. It has a large amount of mycolic acid in the cell wall

B. It appears as a red rod in Gram-stained specimens

C. It appears as a red rod in acid-fast staining

D. It can be detected by light microscopy

E. After being stained with carbolfucshin it resists decolorization with acid

16. On routine physical examination of computer programmer for insurance, a spot is noted on his lungs in the right lobe. He also has been experiencing night sweats, weight lost. What is the most likely cause of disease?

A. Streptococcus pneumonia

B. Mycobacterium tuberculosis

C. Borrelia burgdorferi

D. Pseudomonas aeruginosa

E. Treponema pallidum

17. Which one of the following statements concerning Mycobacterium tuberculosis is NOT correct?

A. Some strains, isolated from individuals with previously untreated cases are resistant to isoniazid

B. It contains a small amount of lipid in a cell wall and stains poorly by gram-stain

C. The organism grow slowly, often requiring 3-6 weeks before colonies appear

D. The antigen in a skin test is a protein extracted from the organism

E. Lowenstein-Jensen medium could be used for organism culture

18. The most common species of Mycobacterium associated isolated from AIDS patients is:

A. Mycobacterium kansasii

B. Mycobacterium leprae

C. Mycobacterium microti

D. Mycobacterium ulcerans

E. Mycobacterium avium-intracellulare

19. Which one of the following pathogenic determinant can be considered a virulence factor of M. tuberculosis?

A. cord factor

B. polysaccharide capsule

C. exotoxin with cytotoxicity

D. LPS

E. enzymes of invasiveness (collagenase, hyaluronidase, protease)

20. BCG vaccine contains:

A. capsular polysaccharide

B. live attenuated M. bovis

C. toxoid

D. live attenuated M. tuberculosis

E. whole killed M. tuberculosis

21. Each of the following statements concerning M. leprae is correct, EXCEPT:

A. In lepromatous leprosy, large numbers of organisms are usually seen in smears stained by acid-fast stain

B. A tuberculoid leprosy case patient is less infective than that with lepromatous leprosy

C. The organism grows will grow well on Lowenstein-Jensen medium in 3 to 6 weeks

D. Skin test with lepromin is useful in diagnosis

E. Some in vivo animals models are currently developed to culture M. leprae

22. A 14 year-old boy developed night sweats, and a low-grade fever. He has shortness of breath and a productive cough with sputum. Acid-fast bacilli have been seen in sputum. Select most appropriate treatment.

A. penicillin

B. rifampin

C. ribavirin and acyclovir

D. streptomycin and lincomycin

E. isoniazid and PAS (paraaminosalicilic acid)

23. Which one of the following statements explains better the use of acid-fast stain, but not Gram-stain procedure in Mycobacterium, tuberculosis staining?

A. It lacks a cell wall and can not retain crystal violet

B. It has a thick polypeptide capsule that does not accept non-concentrated dye

C. It has a large amount of lipids in a cell wall that prevents entry of crystal violet

D. It has a thick peptidoglycan layer which can be decolorized very easy with alcohols

E. It has a large amount of proteins in outer membrane that interfere with staining by c**6.**

24. A patient skin test with purified protein derivative (PPD) to determine previous exposure to Mycobacterium tuberculosis develops induration at the skin test site 48 hours later. Histologically, the reaction site would MOST probably show:

A. Eosinophils

B. Neutrophils

C. B cells

D. NK cells

E. Helper T cells and macrophages

25. Which of the following statements characterizes Mycobacteria tuberculosis better?

A. M. tuberculosis cases strong hypersensitivity reaction type IV in infected patients

B. M. tuberculosis produces highly potent exotoxin

C. M. tuberculosis induces production of protective antibodies in infected patients

D. M. tuberculosis can be effectively killed by macrophages

E. M. tuberculosis grow fast on ordinary media

26. On routine physical examination of computer programmer for insurance, a spot is noted on his lungs in the right lobe. He also has been experiencing night sweats, weight lost. What is the most likely cause of disease?

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B. Mycobacterium tuberculosis

C. Borrelia burgdorferi

D. Pseudomonas aeruginosa

E. Treponema pallidum

27. BCG vaccine contains:

A. Capsular polysaccharide

B. Live attenuated M. bovis

C. Toxoid

D. Live attenuated M. tuberculosis

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28. A 14 year-old boy developed night sweats, and a low-grade fever. He has shortness of breath and a productive cough with sputum. Acid-fast bacilli have been seen in sputum. Select most appropriate treatment.

A. Penicillin

B. Rifampin

C. Ribavirin and acyclovir

D. Streptomycin and lincomycin

E. Isoniazid and PAS (paraaminosalicilic acid)

29. Mycobacteria resist intracellular killing due to which cell wall constituent?

A. Mycolic acids (wax D)

B. Lipopolysaccharide

C. Lipoteichoic acid

D. Peptidoglycan

E. Periplasmic enzymes

30. Each of the following statements concerning Mycobacterium tuberculosis is correct EXCEPT:

A. It has a large amount of mycolic acid in the cell wall

B. It appears as a red rod in Gram-stained specimens

C. It appears as a red rod in acid-fast staining

D. It can be detected by light microscopy

E. After being stained with carbolfucshin it resists decolorization with acid

31. Which one of the following pathogenic determinant can be considered a virulence factor of M. tuberculosis?

A. Cord factor

B. Polysaccharide capsule

C. Exotoxin with cytotoxicity

D. LPS

E. Enzymes of invasiveness (collagenase, hyaluronidase, protease)

32. Each of the following statements concerning M. leprae is correct, EXCEPT:

A. In lepromatous leprosy, large numbers of organisms are usually seen in smears stained by acid-fast stain

B. A tuberculoid leprosy case patient is less infective than that with lepromatous leprosy

C. The organism grows will grow well on Lowenstein-Jensen medium in 3 to 6 weeks

D. Skin test with lepromin is useful in diagnosis

E. Some in vivo animals models are currently developed to culture M. leprae

33. Which one of the following statements explains better the use of acid-fast stain, but not Gram-stain procedure in Mycobacterium, tuberculosis staining?

A. It lacks a cell wall and can not retain crystal violet

B. It has a thick polypeptide capsule that does not accept non-concentrated dye

C. It has a large amount of lipids in a cell wall that prevents entry of crystal violet

D. It has a thick peptidoglycan layer which can be decolorized very easy with alcohols

E. It has a large amount of proteins in outer membrane that interfere with staining by crystal violet

34. Which one of the following statements concerning Mycobacterium tuberculosis is NOT correct?

A. Some strains, isolated from individuals with previously untreated cases are resistant to isoniazid

B. It contains a small amount of lipid in a cell wall and stains poorly by gram-stain

C. The organism grow slowly, often requiring 3-6 weeks before colonies appear

D. The antigen in a skin test is a protein extracted from the organism

E. Lowenstein-Jensen medium could be used for organism culture

35. The most common species of Mycobacterium associated isolated from AIDS patients is:

A. Mycobacterium kansasii

B. Mycobacterium leprae

C. Mycobacterium microti

D. Mycobacterium ulcerans

E. Mycobacterium avium-intracellulare

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D. Skin test with lepromin is useful in diagnosis

E. Some in vivo animals models are currently developed to culture M. leprae

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B. Rifampin

C. Ribavirin and acyclovir

D. Streptomycin and lincomycin

E. Isoniazid and PAS (paraaminosalicilic acid)

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D. It has a thick peptidoglycan layer which can be decolorized very easy with alcohols

E. It has a large amount of proteins in outer membrane that interfere with staining by crystal violet

Recommended reading list

Main literature

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Further Reading

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