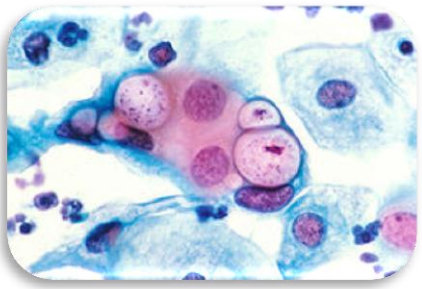
Chlamydiae



Chlamydiae are obligate, aerobic, intracellular parasites of eukaryotic cells. They are small Gram-negative coccoid or rod shaped, non-motile bacteria. *Chlamydiae* exhibit characteristics intermediate between bacteria and viruses.

They are widespread in the natural world, being parasites of people, animals and birds with tropism for squamous epithelial cells and macrophages of the respiratory and gastrointestinal tract.

They are Recognized as Bacteria as:

- They have both DNA and RNA.
- They have cell wall (that resembles that of GNB) and ribosomes
- Replicate by binary fission
- Susceptible to antibiotics

Cell Structure

Chlamydiae have a cytoplasmic membrane and an outer membrane similar to Gram-negative bacteria but lack a peptidoglycan cell wall. *Chlamydiae* cannot synthesize their own ATP and require intracellular abode to remain viable.

Chlamydiae exist in two forms: the elementary body and the reticulate body. Both of them play a pivotal part in the life cycle of chlamydia.

Elementary Body (EB)

The elementary body is the dispersal form, which is analogous to a spore. This dispersal form is about 0.3 μm or 200-300 nm in diameter. It is the extracellular infective form. It induces its own endocytosis upon exposure to target cells.

Reticulate Body (RB)

Reticulate body is the intracellular, multiplicative form. It represents the noninfectious growing form.

Life Cycle

The life cycle of *Chlamydia trachomatis* consists of two stages: elementary body and reticulate body. Upon endocytosis into the host cell. Once inside the endosome, the elementary body transforms into the larger reticulate body (500 – 1000 nm) as a result of the glycogen that is produced. The reticulate body is the reproductive form. It divides through binary fission at approximately 2-3 hours per generation. It contains no cell wall and is detected as an inclusion in the cell arranged as a mantle around the nucleus. The inclusion bodies are basophilic. They can also be stained by Lugol's iodine because of the presence of glycogen matrix. After division, the reticulate body transforms back to the elementary form and is released by the cell by exocytosis. One phagolysosome usually produces 100-1000 elementary bodies. The entire process takes 24 – 48 hours. The EB may infect new cells and the cycle continues.

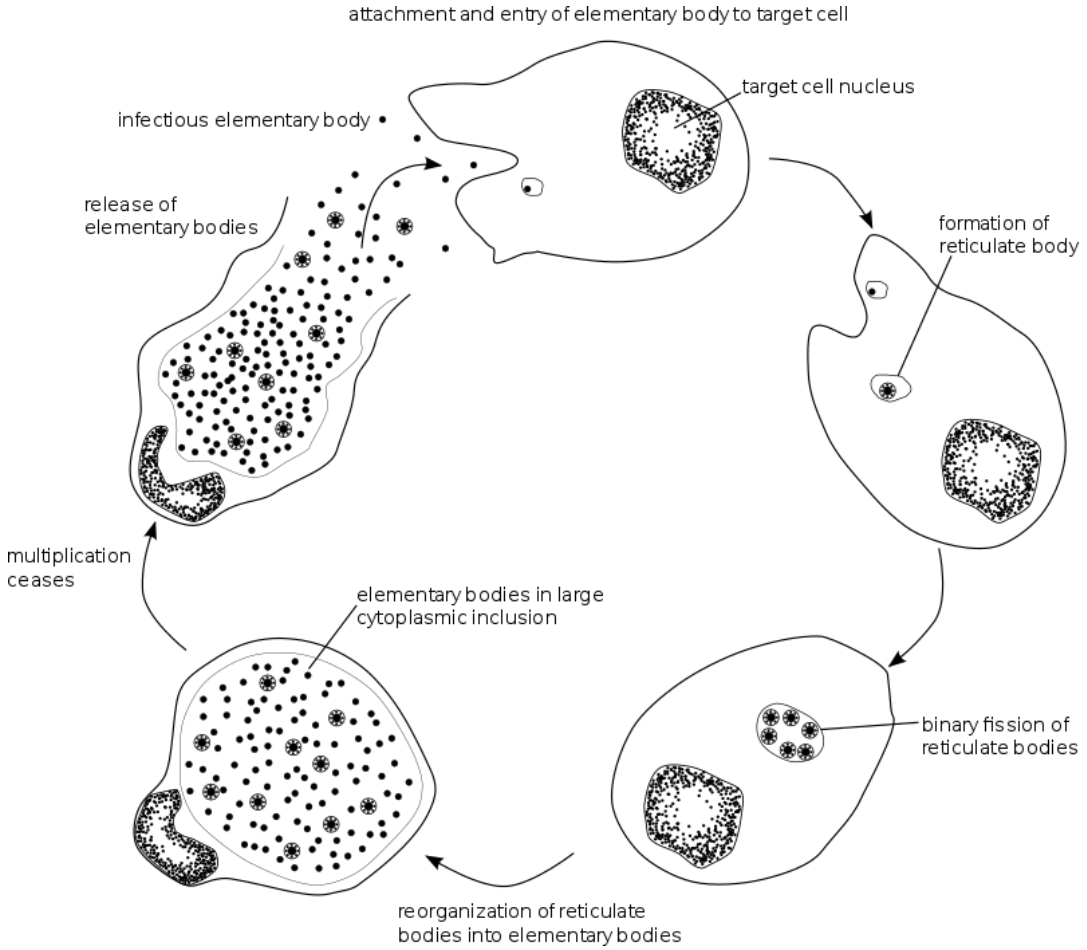


Fig 1: *Chlamydiae* life cycle

Culture

Chlamydiae can be isolated by the following methods:

(a) **Animal inoculation:** Mice can be inoculated through intranasal, intraperitoneal or intracerebral route. Mice die within 10 days. Smears made from lung, spleen, brain or peritoneal exudate demonstrate elementary bodies.

(b) **Egg inoculation:** Organisms can be isolated by egg yolk inoculation of the specimen. Impression smears can be stained by Giemsa or Gimenez.

(c) **Tissue culture:** McCoy cells treated with cycloheximide are the most commonly used cell lines. Irradiated or metabolically inhibited cell lines can also be used for isolation of chlamydia. Inclusion bodies can be visualized by staining the cell lines.

DISEASES PRODUCED BY *CHLAMYDIA*

(a) **Ocular infections:** *C. trachomatis* serotype A,B,Ba,C- is the leading cause of blindness (caused by a chlamydia infection called trachoma) in the world. Other diseases produced are inclusion conjunctivitis (serotype D to K).

(b) **Genital infections:** *C. trachomatis* is also the leading cause of sexually transmitted disease worldwide. It is associated with urethritis and lymphogranuloma venereum . *C. trachomatis* is one of the major causes of pelvic inflammatory disease (PID) and infertility in women.

(c) **Respiratory infections:** *C. pneumoniae* causes pneumonia. *C. psittaci* causes ***psittacosis***.

LABORATORY DIAGNOSIS

***Specimen collection*:** Specimen should be collected by scraping the mucosa. Depending on the site of infection, ocular, urethral, cervical, sputum, respiratory secretions can be collected.

***Direct detection of antigen*:** Antigen detection is a rapid method of diagnosing chlamydial infection.

1. *Light Microscopy:* Inclusion bodies of *C. trachomatis* can be detected by staining with Lugol's iodine. Iodine can be used because inclusion bodies contain a glycogen matrix. Giemsa, Castaneda, Machiavello and Giminez methods are better and can be used to stain ocular, cervical or urethral specimen.
2. *Immunofluoresence:* Direct fluorescent antibody test detects major outer membrane proteins. It is now considered by many the test of choice for diagnosis.

ELISA: Antigen and antibodies can be detected by ELISA. Antigen detection is more specific than antibody detection.

Treatment

Sulphonamides and tetracycline are the drugs of choice. Single dose azithromycin is the drug of choice for urethritis.

Mycobacteria

The mycobacteria are rod-shaped, aerobic bacteria that do not form spores. Although they do not stain readily, after being stained, they resist decolorization by acid or alcohol and are therefore called "acid-fast" bacilli. *Mycobacterium tuberculosis* causes tuberculosis and is a very important pathogen of humans (table-1).

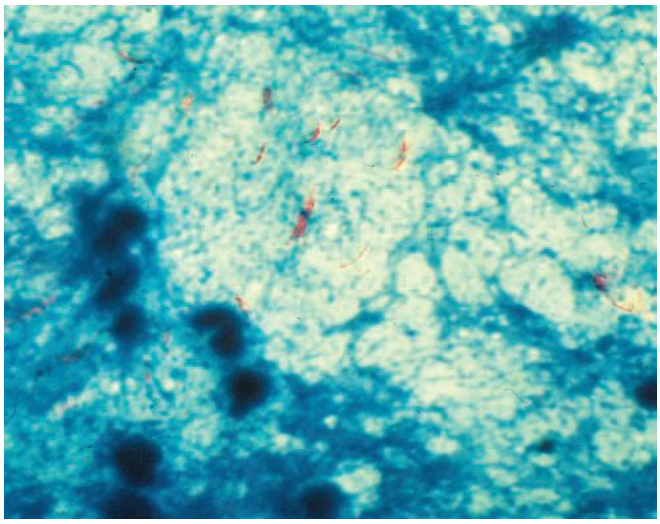
Species	Reservoir	Common Clinical Manifestations; Comment
SPECIES ALWAYS CONSIDERED PATHOGENS		
Mycobacterium tuberculosis	Humans	Pulmonary and disseminated tuberculosis; millions of cases annually in the world
Mycobacterium leprae	Humans	Leprosy
Mycobacterium bovis	Humans, cattle	Tuberculosis-like disease; rare in North America; *M bovis* is closely related to *M tuberculosis*

Mycobacterium tuberculosis

Morphology and Identification:

On artificial media, coccoid and filamentous forms are seen with variable morphology from one species to another. **Mycobacteria cannot be classified as either gram positive or gram negative**. When stained by basic dyes, they cannot be decolorized by alcohol, regardless of treatment with iodine. True tubercle bacilli are characterized

by "acid fastness"—that is, 95% ethyl alcohol containing 3% hydrochloric acid (acid-alcohol) quickly decolorizes all bacteria except the mycobacteria. Acid fastness depends on the integrity of the waxy envelope. The **Ziehl-Neelsen** technique of staining is used for identification of acid-fast bacteria.



Culture

The media for primary culture of mycobacteria should include a nonselective medium and a selective medium. Selective media contain antibiotics to prevent the overgrowth of contaminating bacteria and fungi. There are three general formulations that can be used for both the nonselective and selective media.

1.Semisynthetic agar media: These media (eg, Middle brook 7H10 and 7H11) contain defined salts, vitamins, cofactors, oleic acid, albumin, catalase, and glycerol; the 7H11 medium also contains casein hydrolysate.

2. Inspissated egg media: These media (eg, Lwenstein- Jensen) contain defined salts, glycerol, and complex organic substances (eg, fresh eggs or egg yolks, potato flour, and other ingredients in various combinations). Malachite green is included to inhibit other bacteria.

3. Broth media (eg, Middle brook 7H9 and 7H12) support the proliferation of small inocula.

Growth Characteristics:

Mycobacteria are obligate aerobes and derive energy from the oxidation of many simple carbon compounds.

Reaction to Physical and Chemical Agents

Mycobacteria tend to be more resistant to chemical agents than other bacteria because of the hydrophobic nature of the cell surface and their clumped growth. Dyes (eg, malachite green) or antibacterial agents (eg, penicillin) that are bacteriostatic to other bacteria can be incorporated into media without inhibiting the growth of tubercle bacilli.

Constituents of Tubercle Bacilli

A. Lipids

Mycobacteria are rich in lipids. These include mycolic acids (long-chain fatty acids), waxes, and phosphatides. In the cell, the lipids are largely bound to proteins and polysaccharides. Lipids are to some extent responsible for acid fastness. Their removal with hot acid destroys acid fastness, which depends on both the integrity of the cell wall and the presence of certain lipids.

B. Proteins

Each type of mycobacterium contains several proteins that elicit the tuberculin reaction. Proteins bound to a wax fraction can upon injection, induce tuberculin sensitivity. They can also elicit the formation of a variety of antibodies.

C.Polysaccharides

Mycobacteria contain a variety of polysaccharides. Their role in the pathogenesis of disease is uncertain. They can induce the immediate type of hypersensitivity and can serve as antigens in reactions with sera of infected persons.

Pathogenesis

Mycobacteria are emitted in droplets smaller than 25 μm in diameter when infected persons cough, sneeze, or speak. The droplets evaporate, leaving organisms that are small enough, when inhaled, to be deposited in alveoli. Inside the alveoli, the host's immune system responds by release of cytokines and lymphokines that stimulate monocytes and macrophages. Mycobacteria begin to multiply within macrophages.

Some of the macrophages develop an enhanced ability to kill the organism, but others may be killed by the bacilli.

One to 2 months after exposure, pathogenic lesions associated with infection appear in the lung.

Types of Tuberculosis

Primary Infection and Reactivation

When a host has first contact with tubercle bacilli, the following features are usually observed:

(1) An acute exudative lesion develops and rapidly spreads to the lymphatics and regional lymph nodes. The exudative lesion in tissue often heals rapidly.

(2) The lymph node undergoes massive caseation, which usually calcifies (Ghon lesion).

(3) The tuberculin test result becomes positive.

In primary infections, the involvement may be in any part of the lung but is most often at the base.

The reactivation type is usually caused by tubercle bacilli that have survived in the primary lesion.

Reactivation tuberculosis is characterized by chronic tissue lesions, the formation of tubercles, caseation, and fibrosis. These differences between primary infection and reinfection or reactivation are attributed to (1) resistance and (2) hypersensitivity induced by the first infection.

Immunity and Hypersensitivity

During the first infection with tubercle bacilli, a certain resistance is acquired, and there is an increased capacity to localize tubercle bacilli, retard their multiplication, limit their spread, and reduce lymphatic dissemination. This can be attributed to the development of cellular immunity, with evident ability of mononuclear phagocytes to limit the multiplication of ingested organisms and even to destroy them.