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. Although Gram negative, Chlamydiae stain better with Castaneda, Machiavello or Gimenez stains.

**Elementary body (EB):**

The elementary body is the dispersal form, which is analogous to a spore. This dispersal form is about 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 non-infectious 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 EB prevents phagolysosomal fusion enabling intracellular survival of the bacteria. 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. 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.

Antigenic structure:
Chlamydia antigens consist of 3 groups:
- genus-specific antigen
- species specific protein antigen
- serotype-specific

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.

(c) Tissue culture: 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 preventable blindness (caused by a chlamydia infection called trachoma) in the world. Other diseases produced are inclusion conjunctivitis and ophthalmia neonatorum.

(b) Genital infections: *C. trachomatis* is also the leading cause of sexually transmitted disease worldwide. It is associated with non-gonococcal urethritis and lymphogranuloma venereum. *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. In suspected Psittacosis, blood and sputum are collected for microscopy and culture and serum for serology.

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. **Isolation:** Mice, fertilized hen’s egg and tissue cultures can be used for isolation of chlamydia. The clinical specimen can be inoculated into the yolk sac of 6 to 8 day old eggs.

3. **Immunofluoresence:** Direct fluorescent antibody test detects major outer membrane proteins. It is now considered by many the test of choice for diagnosis.
4. **ELISA:** Antigen and antibodies can be detected by ELISA. Antigen detection is more specific than antibody detection.

5. **Molecular tools:** Polymerase chain reaction, can be used for detection of Chlamydia.

**Treatment:**
Sulphonamides and tetracycline are the drugs of choice. Single dose azithromycin is the drug of choice for non-gonocccocal urethritis.

**Rickettsiaceae:**

Rickettsiae are small, pleomorphic, gram negative bacilli. They are fastidious bacteria that are **obligate intracellular parasites**. They require an **arthropod vector** as part of their natural cycle and are transmitted to man by blood sucking arthropods. They possess both DNA and RNA. They possess a cell wall made of peptidoglycan. They are non motile and non capsulated. They reproduce by binary fission and are susceptible to antibacterial agents. However they are **not visible by light microscopy**.

**Morphology:**

- They are pleomorphic coccobacilli. They possess trilaminar cytoplasmic membrane and cell wall as seen by electron microscopy.

- They are Gram- negative though do not take stain well.

- They stain deep red with Macchiavello and Gimenez while bluish purple with Giemsa and Castaneda stain.

**Cultivation:**

- They are obligate intracellular parasites. They cannot be grown on cell free media. They generally grow in cytoplasm of infected cell but spotted fever rickettsiae grow in nucleus as well. Optimum temperature for growth is 32-35°

  - They can be cultivated in yolk sac of 5-6 days old embryonated egg.
  - They can grow well on continuous cell lines.
  - Mice and guinea pig can be used for primary isolation of rickettsiae from clinical samples.
Antigenic structure:

Rickettsiiae possess 3 types of antigens:

**Group specific soluble antigen:** It is present on surface of organism and is protein in nature.

**Species specific antigen:** It is adherent to the cell and act as adhesin for host cell.

**An alkali stable polysaccharide:** Found in some rickettsiiae and in some non motile strains of Proteus (OX- 19, OX- 2, OX-K). This sharing of antigens forms the basis for Weil- Felix reaction used in diagnosis of rickettsial infections. In this test agglutinins are detected against these Proteus strains.

Pathogenesis:

Man acquire infection by bite or faeces of an infected arthropod vector. On entry into the human body they become localised chiefly in the vascular epithelium leading to thrombus formation.

1- Typhus fever group
   **This consist of:**
   (a) Epidemic (classical) typhus / transmitted by louse
   (b) Brill-Zinsser disease / transmitted by louse
   (c) Endemic typhus/ transmitted by fleas

2- Spotted fever group
   Main vector of spotted fever group are ticks.

3- Rocky Mountain spotted fever: It is the most serious type of infection, transmitted by tick.

4- Rickettsial Pox: Mildest form of rickettsial disease, self-limited, non-fatal. Also known as varicelliform rickettsiosis. Vector is mite.

Diagnosis is carried out by
   (a) Isolation of rickettsiae in lab animals, fertile hen’s egg and cell cultures
   (b) Direct detection of organism and their antigen in clinical samples
   (c) Serology
Isolation of Rickettsiae
Blood clots ground in skimmed milk or BHI broth is inoculated intraperitoneally in guinea pig or mice. Animal will be observed for 3-4 weeks.

Direct detection of organism and their antigen
Aggregates of the organism or their antigen in biopsy specimen from rashes and liver, impression smears from organs of infected animals may be demonstrated by:
- Giemsa staining
- Macciavello staining
- Gimenez staining
- Direct immunoflourescence
- Indirect immunoflourescence
- PCR

Serology
(a) Non-specific reaction:- Weil- Felix Reaction.
(b) Specific:- using rickettsial antigen.

Treatment: Tetracyclines and chloramphenicol can be given to treat rickettsial infection.

Mycoplasma and L-forms
Mycoplasma species are the smallest free-living organisms. These organisms are unique among prokaryotes in that they lack a cell wall, hence lack fixed shape or size and also lack Gram stain reaction and their lack of susceptibility to beta-lactams. Because of their plasticity, they can pass through bacterial filters of 45µm pore size and have often been mistaken for viruses. Mycoplasmal organisms are usually associated with mucosal surfaces of respiratory and urogenital tracts. They rarely penetrate the submucosa, except in the case of immunosuppression or instrumentation, when they may invade the bloodstream and disseminate. Species most commonly associated with infections are Mycoplasma pneumonia, Mycoplasma hominis, and Mycoplasma genitalium.
Pathophysiology:

*M. pneumoniae* causes community-acquired *atypical pneumonia*, tracheobronchitis or bronchiolitis. Pneumonia develops in only 5-10% of persons who are infected. Acute pharyngitis may also occur.

After inhalation of respiratory aerosols, the organism attaches to host epithelial cells in the respiratory tract. It produces adhesions and other accessory proteins which mediate attachment, followed by local inflammation and tissue destruction that may be mediated by liberation of hydrogen peroxide. Recently, *M. pneumoniae* has been shown to produce an exotoxin: *community acquired respiratory disease*. The organism replicates intracellularly, which contribute to chronicity of illness and difficult eradication. Spread of infection throughout households is common. The incubation period is 2-3 weeks.

**Culture:** They can be cultivated on fluid (broth) or solid media (agar) enriched with 20% horse or human serum and yeast extract and addition of antibiotics as selective agents. Colonies appear after incubation for 2-6 days and are about 10-600 μm in size with a typical “fried egg” appearance. Colonies may be seen with a hand lens but are best studied after staining by Dienes method.

**Antimicrobials:**

Oral erythromycin or one of the newer macrolides such as azithromycin or clarithromycin have long been the drug of choice for mycoplasmal respiratory tract infections. Tetracycline and its analogues are also active. As would be predicted by the lack of a cell wall, none of the beta-lactams is effective against *M. pneumoniae*. 

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L-Form Bacteria:

L-form bacteria, also known as L-phase bacteria, and cell wall deficient (CWD) bacteria, are strains of bacteria that lack cell walls.

Two types of L-forms are distinguished: unstable L-forms, spheroplasts that are capable of dividing, but can revert to the original morphology, and stable L-forms. L-forms that are unable to revert to the original bacteria.

L-forms can be generated in the laboratory from many bacterial species that usually have cell walls, such as Bacillus subtilis or Escherichia coli. This is done by inhibiting peptidoglycan synthesis with antibiotics or treating the cells with lysozyme, an enzyme that digests cell walls. Some of the species of L-form bacteria that have been implicated in chronic disease include: *Bacillus anthracis*, *Mycobacterium tuberculosis*, *Treponema pallidum*, and *Rickettsia prowazekii*. Although L-forms can develop from Gram-positive as well as from Gram-negative bacteria, in a Gram stain test, the L-forms always colour Gram-negative due to the lack of a cell wall.