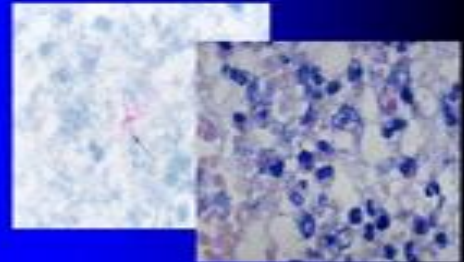


BACTERIA WITH ATYPICAL CELL WALLS

- ❑ Not all bacteria are classified based on gram staining
- ❑ Some bacterial groups lack typical cell wall structure and may stain poorly with gram stain
 - Mycobacterium
 - cell wall has lipid called **mycolic acid**
 - basis for **acid-fast stain**
- ❑ Some have no cell wall
 - Mycoplasma
- ❑ Flexible thin walled bacteria (spirochetes)
 - Treponema sp
 - Leptospira sp
 - Borrelia sp
- ❑ Obligate intracellular bacteria
 - Chlamydia sp
 - Rickettsia sp

Acid Fast Stain



The mycoplasmas are essentially bacteria lacking a rigid cell wall during their entire life cycle, although they are also much smaller than bacteria. The first organism of this type was associated with pleuropneumonia of cattle, and was originally called the pleuropneumonia organism (PPO)

Diseases Caused by Mycoplasma

<u>Organism</u>	<u>Disease</u>
<i>M. pneumoniae</i>	Upper respiratory tract disease, tracheobronchitis, atypical pneumonia, (chronic asthma??)
<i>M. hominis</i>	Pyleonephritis, pelvic inflammatory disease, postpartum fever
<i>M. genitalium</i>	Nongonococcal urethritis
<i>U. urealyticum</i>	Nongonococcal urethritis, (pneumonia and chronic lung disease in premature infants??)

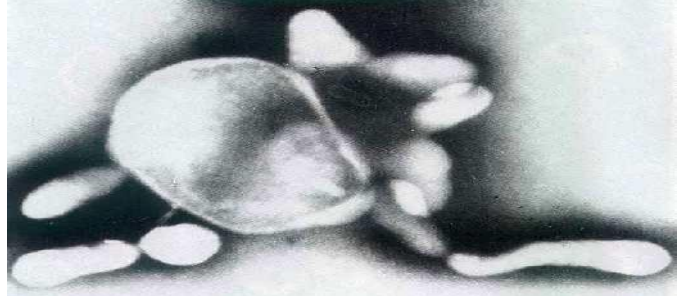
N.B. Other organisms infect humans but their disease association is not known.

Morphology

-The smallest among prokaryotic microorganisms with circular dsDNA, usually 0.2-0.3µm in size; lack of cell wall;

-Pleomorphic, spherical, short rod, filament; Gram negative, but stained hardly, usually use Giemsa stain. ■

-They can assume multiple shapes including round, pear shaped and even filamentous.



General Characteristics

1-smallest known free-living organisms.

2-Because of the absence of cell walls, they do not stain with the Gram stain, and they are more pleomorphic and plastic than eubacteria.

3-**Giemsa stain**

4-they appear as tiny pleomorphic cocci, short rods, short spirals, and sometimes as hollow ring forms. Their diameter ranges from 0.15 μ to 0.30 μ .

5=very small (0.2 x 0.8 μ m)

6- pass through a 0.45 μ m filter

7-No Cell wall: plasma membrane only

8-resistant to antibiotics that interfere with the integrity of cell wall; penicillins, cephalosporins, vancomycin, bacitracin

9-susceptible to tetracycline, erythromycin

Structure

-The cell is enclosed by a limiting membrane which is more similar to that of animal cells than that of bacterial cells because of sterols present in the membrane.

-The cytoplasm contains ribosomes, but lacks mesosomes. There is no nuclear membrane.

-requires sterols for growth, can be grown on laboratory media

-most are facultatively anaerobic Exception *M. pneumoniae*

-Pneumonia caused by *Mycoplasma pneumoniae* usually named walking pneumonia frequently confused with virus infection it is primary atypical

Mycoplasma pneumoniae Laboratory Diagnosis

- Specimens: but scanty sputum
- Gram stain : not useful.
- Culture: on **special bacteriologic** media. Takes at least 10 days to grow (too long to be clinically useful).
 - Colonie: Fried egg appearance

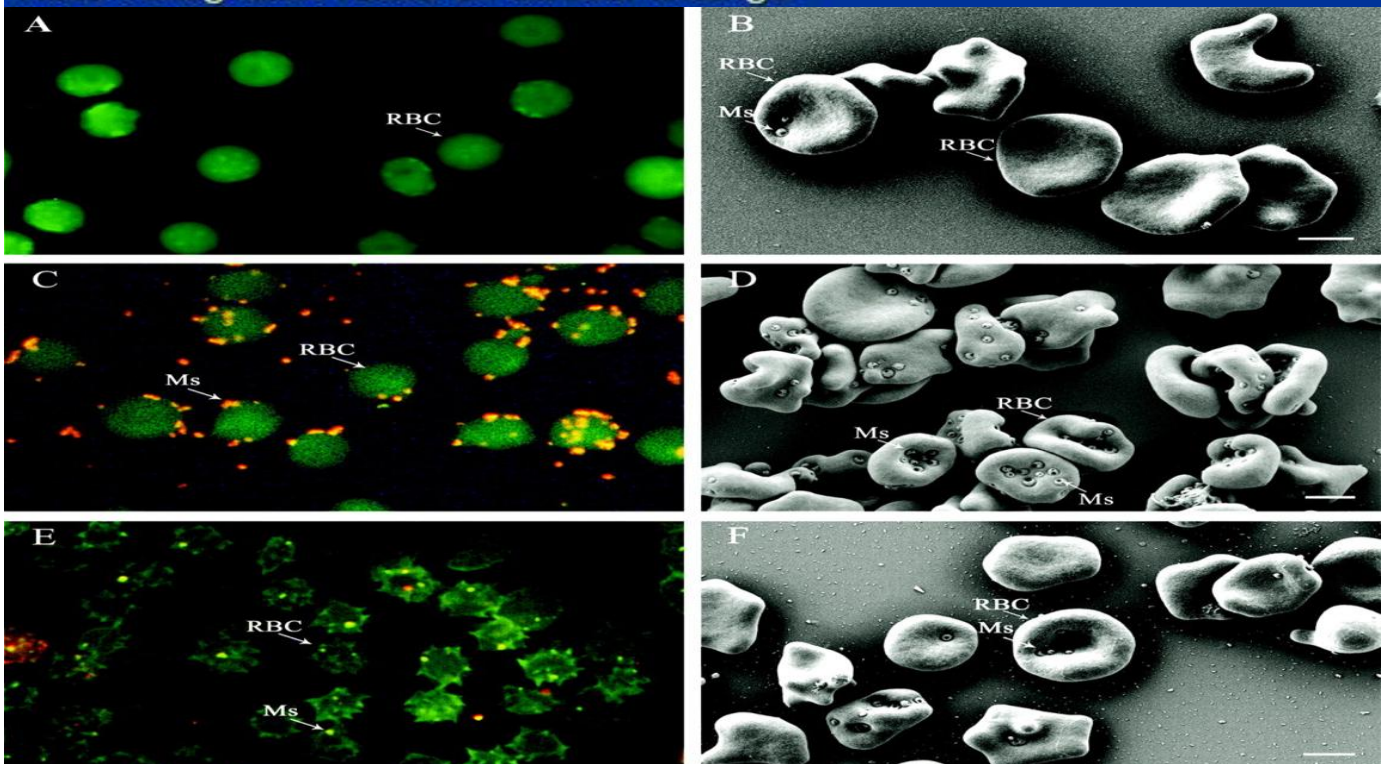


Serology

- A cold-agglutinin titer of 1:128 or higher is indicative of recent infection
 - Cold agglutinin: IgM autoantibodies against red blood cells that agglutinate these cells at 4 °C but not at 37 °C
- Complement fixation test for antibodies to *Mycoplasma pneumoniae* is more specific.

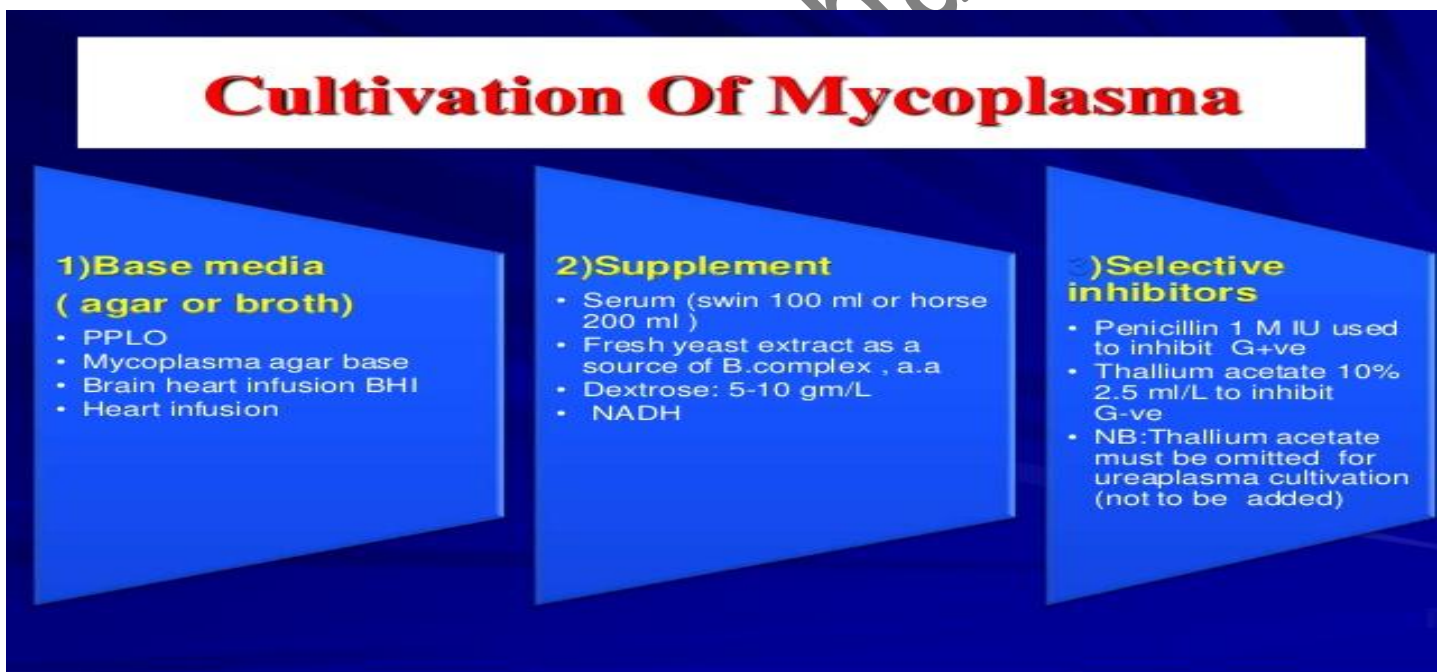
PCR

Note : Diagnosis relies on clinical findings



Culture

- Culture Mycoplasma from sputum, mucous membrane swabbings or other specimens
- direct inoculation into liquid or solid media containing serum, yeast extract and penicillin to inhibit contaminating bacteria.
- Most aerobic; require 10%-20% human or animal serum added to basic nutrient media except *Acholeplasma*; typical colony show fried egg appearance.
- Many species are part of the normal flora
- These organisms are **a frequent cell culture contaminant**
- The organisms have limited biosynthetic abilities; they require cholesterol for their cell membrane and can generate energy via the breakdown of arginine
- Ureaplasma* requires urea to produce an electrochemical gradient; urea is converted to ammonia to produce ATP.



Cultural Characteristics

- Despite the lack of a cell wall, they do not require a medium of very high osmotic pressure.
- **On solid media**, they form minute, transparent colonies.
- looks like a fried egg. The different strains vary in their growth rate
- may take from two days to several weeks to form a colony.

"fried egg"
colonial
morphology.

Fried Egg Colonies



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serology

1: complement fixation

- on acute and convalescent serum.
- patient's serum heated to 56C to eliminate complement
- combine patient's serum and known Mycoplasma antigen in presence of added - complement. Mix.
- Incubate - add indicator system

Red cells and anti-red cell antibody

- hemolysis occurs if complement is unused

2-Hemagglutination

- Cold agglutinins to human O erythrocytes.
- hemabsorption & B-hemolysis of guinea pig red blood cells

M. pneumoniae Nucleic Acid Probes

specific recombinants to oligonucleotide sequences that are only found in Mycoplasma pneumoniae

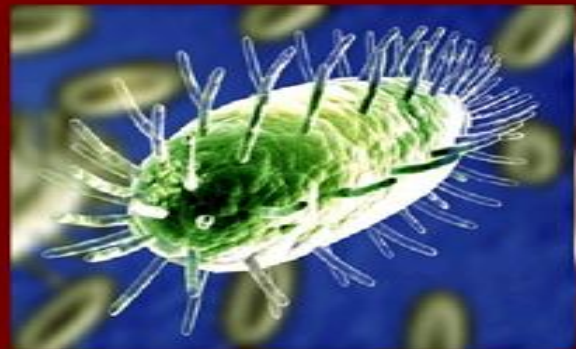


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How they differ from Viruses

- They grow on cell-free media in vitro.
- They contain both **RNA and DNA**.
- They have both intracellular and extracellular parasitism in vivo.



How they differ from other Bacteria

- They have sterols in the cell membrane.
- They share no DNA homology with known bacteria.
- They have low guanine levels plus cytosine content.
- Their genome has a low molecular weight.
- They exhibit no reversion to walled forms.

Classifications

Typical

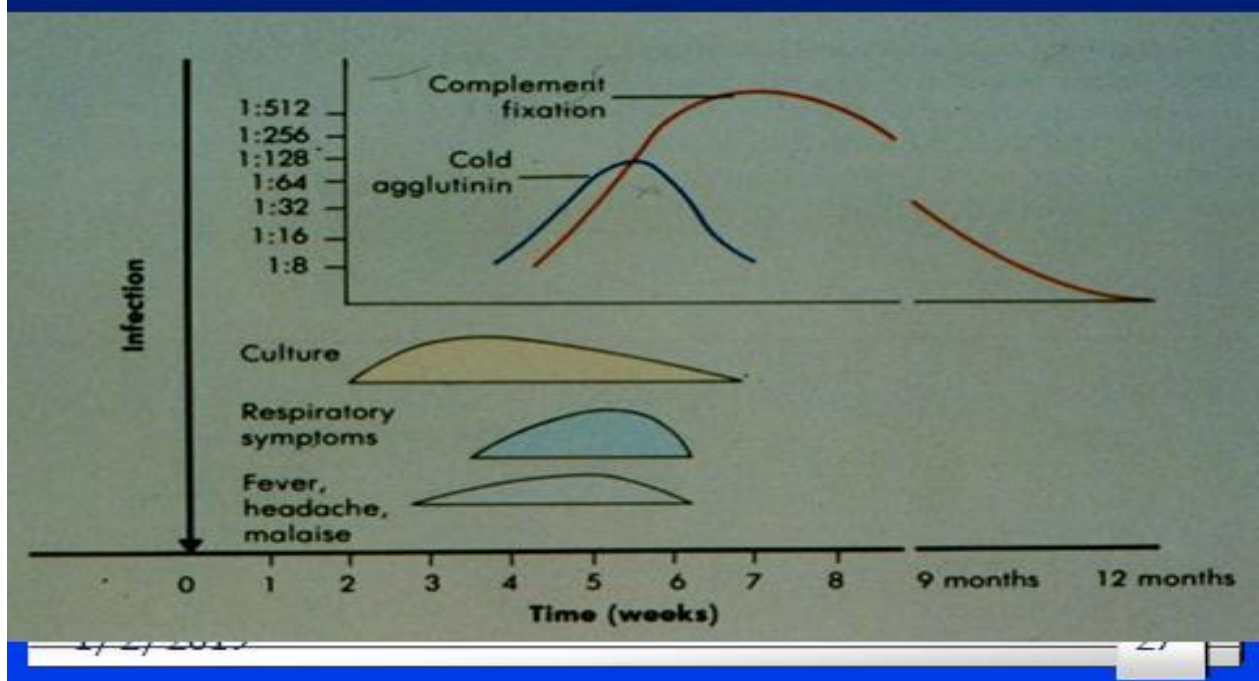
- Typical pneumonia usually is caused by bacteria
- *Strept. Pneumoniae*
– (lobar pneumonia)
- *Haemophilus influenzae*
- Gram-negative organisms
- *Moraxella catarrhalis*
- *S. aureus*

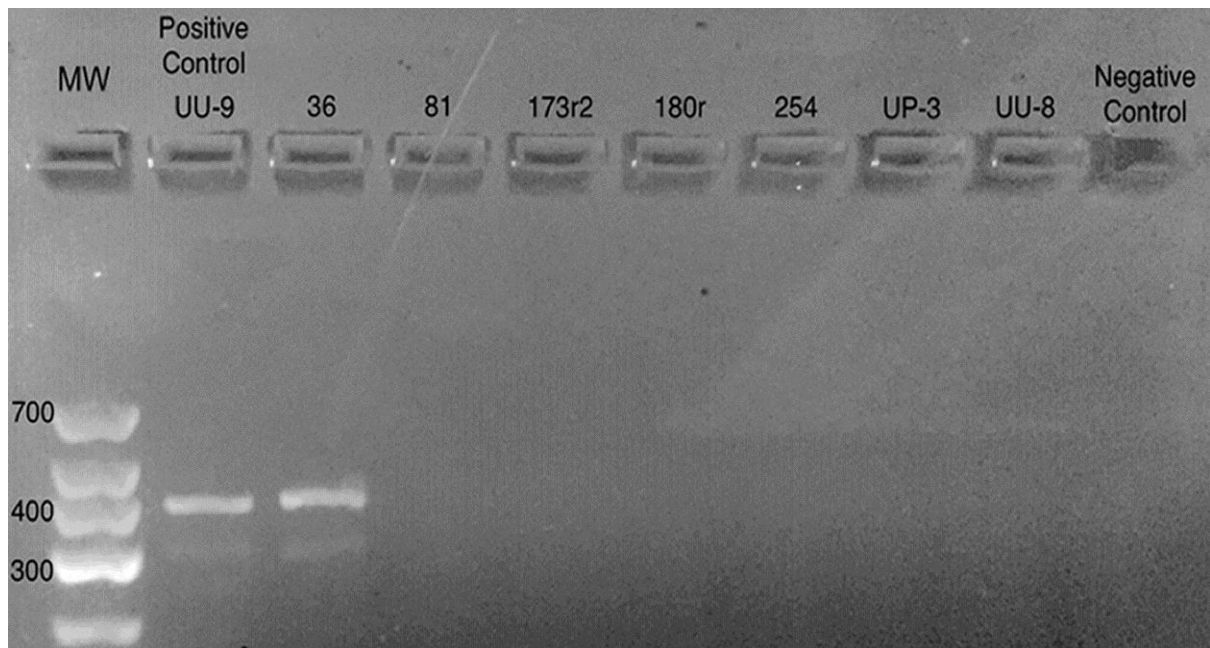
Atypical

- Atypical: not detectable on gram stain; won't grow on standard media
- *Mycoplasma pneumoniae*
- *Chlamydia pneumoniae*
- *Legionella pneumophila*
- *Influenza virus*
- *Adenovirus*
- TB
- Fungi

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In some strains, amorphous material on the outer surface of the membrane suggests the existence of a capsule