

MYCOPLASMA & UREAPLASMA

General Features

- Smallest microbes capable of free-living in the environment and self-replicating on artificial culture media
- Very small (150–350 nm), filterable
- Can grow on artificial cell-free culture media
- **lack a rigid cell wall, which is replaced by a triple layered cell membrane containing sterol** →
 - **Pleomorphic** - coccoid, bacillary or filamentous
 - Completely resistant to antibiotics acting on cell wall such as β -lactam

General Features

- poorly gram-negative, better stained by Giemsa stain
- reproduce by binary fission and budding
- non-sporing and non-flagellated, usually nonmotile.
- However, **gliding motility**
- **Contaminants of cell cultures**

Classification

- Family Mycoplasmataceae comprises of two genera—
(1) *Mycoplasma* and (2) *Ureaplasma*
- **Human pathogenic species are:**
 - *Mycoplasma pneumoniae* causing pneumonia
 - Others cause genital tract infections:
 - ☐ *Mycoplasma hominis*
 - ☐ *Mycoplasma genitalium*
 - ☐ *Ureaplasma urealyticum*
 - ☐ *Ureaplasma parvum*.

MYCOPLASMA PNEUMONIAE

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- **Antigens**
- *cell membrane antigens*
- **Glycolipid antigen: Nonspecific, found in diverse tissues – basis heterophile antibody tests (cold agglutination test)**
- **Membrane bound proteins** (e.g. cytoadhesin P1 protein): help in attachment to host cell surface

Pathogenesis

- **Adhesion:** to respiratory mucosa - most important step
- **Injury to host respiratory tissue by**
 - Hydrogen peroxide
 - **Cytotoxin:** ADP ribosylating and vacuolating properties similar to pertussis toxin
 - **Lipoproteins:** present in the cell membrane and appear to induce inflammation.

Host Immunity

- **Cellular immunity:** immunopathogenic role, exacerbating pneumonia caused by *Mycoplasma*
- **Humoral immunity:**
 - Does not protect from early disease
 - Protection against disseminated *Mycoplasma infections* such as arthritis, meningitis, and osteomyelitis

Epidemiology

- Infection occurs worldwide
- **Transmission:** respiratory droplets expectorated during coughing
- **Facilitating factors:** close contacts as in families, military bases, boarding schools, and summer camps
- Endemic infection, with periodic epidemics every 4–7 years
- Incubation period :2–4 weeks.

Clinical Manifestations

- **Upper Respiratory Tract Infections (URTI)**
 - Pharyngitis, tracheobronchitis or rarely otitis media
 - Acute in onset and 20 times more common than pneumonia
- ***“Atypical” community acquired interstitial pneumonia***
 - Also called as Eaton agent pneumonia, primary atypical pneumonia and walking pneumonia
 - Gradual onset, wheeze or rales, dry cough and peribronchial pneumonia with thickened bronchial markings and streaks of interstitial infiltration on chest X-ray.

Atypical pneumonia

- **Interstitial space** is infected in contrast to typical pneumonia which involves the alveoli.
- Manifests as **non-productive dry cough** whereas, productive cough with purulent sputum is characteristic of alveolar pneumonia
- **Other agents causing Atypical pneumonia**

☒ *M. pneumoniae*

☒ *Chlamydophila pneumoniae*

☒ *Legionella pneumophila*

☒ Viral pneumonia

Extrapulmonary Manifestations

- Result of active *Mycoplasma* infection (e.g. septic arthritis)
- Postinfectious autoimmune phenomena (e.g. Guillain–Barre syndrome).
- **Neurologic:** Meningoencephalitis, encephalitis, Guillain- Barre syndrome and aseptic meningitis
- **Dermatologic:** Skin rashes including erythema multiforme major (Stevens–Johnson syndrome)
- **Cardiac:** Myocarditis, pericarditis
- **Rheumatologic:** Reactive arthritis
- **Hematologic:** Anemia and hypercoagulopathy

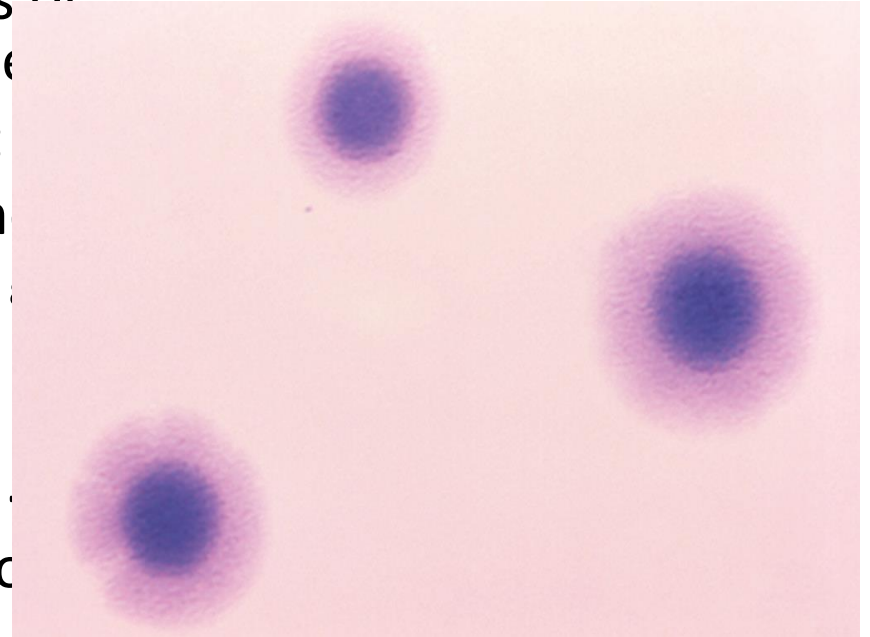
Laboratory Diagnosis

- **Specimen Collection and Transport**
 - Throat swabs and nasopharyngeal aspirates, bronchial brushing, bronchoalveolar lavages and lung biopsies.
 - Sputum is not very useful as it contains too many contaminants
 - Transport media:
- **Standard *Mycoplasma fluid medium*** containing fetal bovine serum, gelatine and penicillin
- **Viral transport medium**, added with ampicillin and cefotaxime.

Culture

- Primary isolation requires complex media:
- **Standard solid medium:** Containing PPLO agar, horse serum and penicillin
- **Standard liquid medium:** Containing PPLO broth, glucose and penicillin and phenol red (indicator)
- **Diphasic medium:** Contains both standard solid phase and liquid phase media as described above
- **SP-4 medium:** It is more complex and contains fetal bovine serum
- **Hayflick modified medium:** Containing heart infusion broth.

- Incubated at 37°C for 5–7 days or sometimes even up to 1–3 weeks
- Growth is detected as follows:
- **In liquid medium:** turbidity and red colour due to fermentation of urea
- **In solid medium:** colonies
- **Shape:** fried egg colonies
- **Size:** Colony size 200–500 μm for mycoplasmas and 15–60 μm for ureaplasmas



Identification

- **Colonies can be examined by:** Hand lens or
- Dienes' staining: alcoholic solution of methylene blue and azure – Mycoplasma intense royal blue, ureaplasmas appear reddish to greenish-blue
- **Identification**
- Hemolytic colonies
- **Phemadsorption test:** *Agglutinates* guinea pig RBCs and the colonies on agar adsorb RBCs to their surface
- **Tetrazolium reduction test**
- **Growth inhibition test:** Inhibited by adding specific antisera

Antigenic Detection

- Direct immunofluorescence test: Detects antigens directly in clinical specimens
- Capture ELISA - antibodies against P1 adhesion antigen
- **Antibody Detection in Serum**
 - Detected after about 1 week of illness. Peak at 3–6 weeks
 - Serological assays have sensitivity and specificity of 55–100%.
- IgM antibodies in children
- IgA-antibody adults

Nonspecific Antibody Detection Tests

- Heterophile antibodies
- **Cold agglutination test:** It uses human O blood group RBC ('I' antigen) and test is carried out at 4°C
- **Streptococcus MG tests:** It uses killed suspension of *Streptococcus MG (group F Streptococcus)*.
- Less commonly used nowadays – poor sensitivity and specificity (positive only in 30–50% of cases).

Specific antibody detection tests

- **Complement fixation test (CFT):** It detects antibodies to glycolipid antigen. A reference test in the past now not in use
- **Alternative techniques with greater sensitivity are:**
 - ☐ Immunofluorescence assays
 - ☐ Latex agglutination assays
 - ☐ ELISA using protein P1 antigens.

Molecular Methods

- **PCR** targeting *M. pneumoniae* specific 16S rRNA gene and P1 adhesion gene is available (sensitivity of 65–90% & specificity of 90–100%)
- **Multiplex PCR for atypical pneumonia**—*M. pneumoniae*, *Chlamydophila pneumoniae* and *Legionella pneumophila*
- **Real-time PCR**: quantitative detection of *M. pneumoniae*.

Treatment *Mycoplasma pneumoniae*

- **Macrolides** (oral azithromycin, 500 mg on day 1, then 250 mg on days 2 to 5)
- Alternative drugs : Doxycycline, Respiratory fluoroquinolones such as levofloxacin, moxifloxacin and gemifloxacin (not ciprofloxacin).

URO GENITAL MYCOPLASMAS

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- *M. hominis, M. genitalium, Ureaplasma (U. urealyticum, and U. parvum)* are associated with urogenital tract disease.
- They frequently colonize female lower urogenital tract such as vagina, periurethral area and cervix
- Transmission: They are transmitted mostly by sexual contact or mother to fetus during birth

Clinical Manifestations

- Non-gonococcal urethritis and epididymitis (mainly due to *Ureaplasma* and *M. genitalium*)
- Pyelonephritis (*M. hominis*), & urinary calculi (*Ureaplasma*)
- Pelvic inflammatory disease (mainly due to *M. hominis*)
- Postpartum and postabortal infection
- Non-urogenital infections (rare, due to *M. hominis*) such as: Brain abscess, wound infections or neonatal meningitis.

Laboratory Diagnosis

- Culture and PCR are the appropriate methods for diagnosis of urogenital mycoplasmas.
- *Ureaplasma* forms very tiny colonies of 15–50 μm size -previously named as T-form *Mycoplasma*.
- **Treatment *Mycoplasma pneumoniae***
 - Macrolides (azithromycin) are the drug of choice for *Ureaplasma* and *M. genitalium* infections
 - Doxycycline is the drug of choice for *M. Hominis*